Lung Biology in Health and Disease Volume 220
Executive Editor: Claude Lenfant

Narcolepsy and Hypersomnia

edited by
Claudio L. Bassetti
Michel Billiard
Emmanuel Mignot

VISIT…

Narcolepsy and Hypersomnia

Edited by

Claudio L. Bassetti
University Hospital
Zurich, Switzerland

Michel Billiard
Gui de Chauliac Hospital
Montpellier, France

Emmanuel Mignot
Stanford University School of Medicine
Stanford, California, U.S.A.

informa
healthcare

New York London

Informa Healthcare USA, Inc.
270 Madison Avenue
New York, NY 10016

© 2007 by Informa Healthcare USA, Inc.
Informa Healthcare is an Informa business

No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-3715-1 (Hardcover)
International Standard Book Number-13: 978-0-8493-3715-4 (Hardcover)

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Reasonable efforts have been made to publish reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

No part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC) 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Library of Congress Cataloging-in-Publication Data

Narcolepsy and hypersomnia / Claudio L. Bassetti, Michel Billiard, Emmanuel Mignot.
p. ; cm. -- (Lung biology in health and disease ; v. 220)
Includes bibliographical references and index.
ISBN-13: 978-0-8493-3715-4 (hardcover : alk. paper)
ISBN-10: 0-8493-3715-1 (hardcover : alk. paper)
1. Narcolepsy. 2. Hypersomnia. I. Bassetti, Claudio, L. II. Billiard, M. (Michel) III. Mignot, Emmanuel. IV. Series.
[DNLM: 1. Narcolepsy. 2. Hypersomnolence, Idiopathic. 3. Kleine-Levin Syndrome. W1 LU62 v.220 2007 / WM 188 N222 2007]

RC549.N37 2007
616.8'498--dc22 2006049753

Visit the Informa Web site at
www.informa.com

and the Informa Healthcare Web site at
www.informahealthcare.com

Introduction

From a medical viewpoint, narcolepsy (and its corollary hypersomnia) is a very interesting disease, not to say a fascinating one. It is also a mysterious disease which may not receive the recognition it deserves, unless of course one is affected by it! Nonetheless, the medical history of this disease is already long and has been superbly traced by one of the editors of this monograph (1). Briefly, the first description of the symptoms was reported in 1877 in Germany, but the name "narcolepsy" was given by a French physician in 1880. Interestingly, the name is derived from Greek, and it means "seized by somnolence." However, it is only in the mid-nineteen hundreds that the tetrad of the disease was described: excessive daytime sleepiness, cataplexy, sleep paralysis, and hypnagogic hallucinations.

Perhaps one of the most intriguing aspects of the evolution of our knowledge of narcolepsy is that it was first thought to be some sort of psychological disorder or an escape from reality. Eventually it was recognized that it is a somatic disease. However, many questions emerged regarding the cause: Is it a neurochemical dysfunction? Is it an autoimmune disorder? Is it a genetic condition? The reality is that each of these determinants is playing a role in the development of the disease. As often happens in medical sciences, it is research on the possible treatment of another disease, obesity, which led to the discovery of two peptides expressed in the hypothalamus and named "hypocretins." The discovery of these peptides and of their receptors opened the door to the most current understanding of narcolepsy.

This volume, *Narcolepsy and Hypersomnia*, does not complete the journey of narcolepsy, but it gives the most complete and up-to-date presentation of the disease, its manifestations, its pathogenetic pathways, and its current treatments.

Narcolepsy is not a rare disease. All over the world, it affects thousands of patients who will eventually benefit from the work of the many experts working on it. The editors, Drs. Claudio Bassetti, Michel Billiard, and Emmanuel Mignot deserve much commendation for organizing this book and calling on international experts. In many ways, this volume superbly illustrates the reasons for the initiation of this series of monographs, Lung Biology in Health and Disease: to inform, to educate, and to stimulate!

As the chief editor of this series, I want to express my thanks and appreciation to the editors and contributors for the privilege to introduce the volume to our readership.

Claude Lenfant
Gaithersburg, Maryland, U.S.A.

Reference

1. Mignot E. A hundred years of narcolepsy research. Archives Italiennes de Biologie 2001;139:207–220.

Preface

It has been almost 30 years since the First International Symposium on Narcolepsy was held in La Grande Motte (France) in 1975, under the leadership of William C. Dement, Christian Guilleminault and Pierre Passouant. In this first symposium, a milestone in the area of narcolepsy, the basis of the questions we are still exploring today were laid out. It was recognized that narcolepsy symptoms were intimately related to rapid eye movement (REM) sleep abnormalities. A natural animal model of narcolepsy, canine narcolepsy, was first reported. The first epidemiological and family studies of the condition were described. New classes of pharmacological agents including tricyclics and gammahydroxybutyrate were found to be useful in the treatment of cataplexy, leading to a better codification of narcolepsy therapies.

The discovery of the HLA-narcolepsy association in 1983 rekindled interest in the condition and raised the possibility of immune abnormalities in the disorder. Several international symposia on narcolepsy were then held, including one at Stanford (USA) in 1985, one in Oak Park (USA) in 1989, one in Paris (France) in 1993 and one in Tokyo (Japan) in 1994.

In 1999, the positional cloning of the canine narcolepsy gene and its identification as the hypocretin (orexin) receptor 2 gene was another milestone in the field. A mouse knockout model for the hypocretin gene was also found to display narcolepsy-like symptoms. In 2000, these discoveries were followed by the report that most cases of human narcolepsy-cataplexy are associated with hypocretin deficiency. Together with the HLA association, these results suggest that narcolepsy may be an autoimmune disorder targeting hypocretin-containing cells in the hypothalamus.

These discoveries are leading to new diagnostic procedures, for example the measurement of cerebrospinal fluid hypocretin-1 levels, and have rekindled research

interest in brain mechanisms hypersomnia. New animal models and novel therapeutic strategies targeting the immune or the hypocretin systems are being developed. Improved epidemiological surveys, a better definition of the narcolepsy spectrum, the finding of hormonal and metabolic abnormalities in narcolepsy, the identification of non-HLA genes involved in narcolepsy are other areas under active investigation.

The explosion of research in the area of narcolepsy and hypocretin mandated the need for an international body to meet, discuss and report on these new developments. Switzerland, a country with a long tradition in sleep research and medicine, was chosen for this event. The event will take place at the Centro Stefano Franscini in the serene and picturesque surroundings of Monte-Verità, Ascona (Ticino, Switzerland).

In the spirit of communicating the great changes that have occurred in the field, we felt it was time to publish an updated monograph reporting on Narcolepsy and Hypersomnia. We took great care in inviting leading experts who could cover all aspects of narcolepsy and hypersomnia in a comprehensive textbook to be used by clinicians and researchers alike as a reference book for many years to come. We hope you will enjoy the resulting book, *Narcolepsy and Hypersomnia*, published by Informa Healthcare and the series editor Claude Lenfant.

Claudio L. Bassetti
Michel Billiard
Emmanuel Mignot

Contributors

M. Abe Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

Peter Achermann Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland

Viola Arias Stanford University Sleep Disorders Program, Stanford, California, U.S.A.

Isabelle Arnulf Fédération des Pathologiés du Sommeil, Hôpital Pitié-Salpêtriére, Public Assistance, Hôpitaux de Paris, France

Claudio L. Bassetti University Hospital, Zurich, Switzerland

Christian R. Baumann University Hospital, Zurich, Switzerland

Pierre Beitinger Max Planck Institute of Psychiatry, Munich, Germany

Michel Billiard Gui de Chauliac Hospital, Montpellier, France

Jed Black Stanford University, Sleep Disorders Clinic, Stanford, California, U.S.A.

John L. Black Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Bastiaan R. Bloem Department of Neurology, Radboud University, Nijmegen Medical Center, Nijmegen, The Netherlands

Roger J. Broughton Ottawa Health Research Institute, Ottawa Hospital and University of Ottawa, Ottawa, Canada

Bertrand Carlander Department of Neurology, Gui de Chauliac Hospital, Montpellier, France

James Allan Cheyne Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada

Thien Dang-Vu Department of Neurology, Université de Liége, Cyclotron Research Center, Liege, Belgium

Yves Dauvilliers CHU Montpellier, Unité de Sommeil Hôpital Gui de Chauliac, Montpellier, France

William C. Dement Stanford University School of Medicine, Palo Alto, California, U.S.A.

Christine Erhardt Service d'Explorations Fonctionnelles, du Système Nerveux et de Pathologie du Sommeil Place de l'Hôpital, Strasbourg, France

Juliette Faraco Stanford University School of Medicine, Stanford, California, U.S.A.

Franco Ferrillo Department of Motor Sciences, Center for Sleep Medicine-DISMR, University of Genova, S. Martino Hospital, Genova, Italy

Laurel Finn University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

Paul A. Fredrickson Department of Psychiatry and Psychology and Sleep Disorders Center, Mayo Clinic College of Medicine, Jacksonville, Florida, U.S.A.

Christian Guilleminault Stanford University Sleep Disorders Program, Stanford, California, U.S.A.

Christian W. Hess Department of Neurology, University Hospital, Bern, Switzerland

Yasuo Hishikawa Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

Yutaka Honda Neuropsychiatric Research Institute, Tokyo, Japan

Christer Hublin Finnish Institute of Occupational Health, Brain@Work Research Center, Helsinki, Finland

T. Kanbayashi Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

Minae Kawashima Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Karl Kesper Sleep Laboratory of the Department of Pneumology, Philipps-University, Marburg, Germany

Ramin Khatami Department of Neurology, University Hospital, Zurich, Switzerland

Thomas S. Kilduff Biosciences Division, SRI International, Menlo Park, California, U.S.A.

Lyudmila I. Kiyashchenko Department of Psychiatry and Biobehavioral Science, Brain Research Institute University of California, Los Angeles, California, U.S.A.

Lois E. Krahn Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Scottsdale, Arizona, U.S.A.

Jean Krieger Service d'Explorations Fonctionnelles, du Système Nerveux et de Pathologie du Sommeil Place de l'Hôpital, Strasbourg, France

Gert Jan Lammers Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

Hans-Peter Landolt Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland

Luis de Lecea Departments of Molecular Biology and Neuropharmacology, The Scripps Research Institute, La Jolla, California, U.S.A.

Ji Hyun Lee Stanford University Sleep Disorders Program, Stanford, California, U.S.A.

Ling Lin Stanford University School of Medicine, Stanford, California, U.S.A.

Johannes Mathis Department of Neurology, University Hospital, Bern, Switzerland

Geert Mayer Hephata Klinik, Schwalmstadt-Treysa, Germany

Emmanuel Mignot Stanford University School of Medicine, Stanford, California, U.S.A.

Boris Y. Mileykovskiy Department of Psychiatry and Biobehavioral Science, Brain Research Institute University of California, Los Angeles, California, U.S.A.

Merrill M. Mitler National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville, Maryland, U.S.A.

Jacques Montplaisir Centre d'Étude du Sommeil, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada

Stephen R. Morairty Biosciences Division, SRI International, Menlo Park, California, U.S.A.

Michael Mühlethaler C.M.U. Département de Physiologie, Geneva, Switzerland

Brian J. Murray Sunnybrook and Women's College Hospital, University of Toronto, Toronto, Canada

Soňa Nevšímalová Department of Neurology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Agnes Nicolet School of Pharmacy, Montpellier, France

Seiji Nishino Stanford University School of Medicine, Stanford, California, U.S.A.

Lino Nobili Department of Motor Sciences, Center for Sleep Medicine-DISMR, University of Genova, S. Martino Hospital, Genova, Italy

Maurice M. Ohayon Stanford Sleep Epidemiology Research Center, School of Medicine, Stanford University, Stanford, California, U.S.A.

Laurence K. Oliver Sleep Disorders Center, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Cecilia Orellana Hospital de Clinicas, Instituto de Neurologia, Montevideo, Uruguay

Sebastiaan Overeem Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen and Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

Covadonga Pañeda Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, U.S.A.

Daniel Pardi Jazz Pharmaceuticals, Inc., Palo Alto, California, U.S.A.

Thomas Penzel Sleep Laboratory of the Department of Pneumology, Philipps-University, Marburg, Germany

Christelle Peyron CNRS UMR 5167, Institut Fédératif des Neurosciences de Lyon, Université Claude Bernard-Lyon 1, Lyon, France

Hanno Pijl Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands

Thomas Pollmächer Center of Mental Health, Klinikum Ingolstadt, Ingolstadt, Germany

Sylvie Rompré Centre d'Étude du Sommeil, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada

Takeshi Sakurai Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan and ERATO Yanagisawa Orphan Receptor Project, Japan Science and Technology Corporation, Tokyo, Japan

Thomas E. Scammell Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, U.S.A.

Carlos H. Schenck Department of Psychiatry, Minnesota Regional Sleep Disorders Center, Hennepin County Medical Center, University of Minnesota Medical School, Minneapolis, Minnesota, U.S.A.

Andreas Schuld Center of Mental Health, Klinikum Ingolstadt, Ingolstadt, Germany and Max Planck Institute of Psychiatry, Munich, Germany

Tetsuo Shimizu Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

Adrian M. Siegel Department of Neurology, University Hospital, Zurich, Switzerland

Jerome M. Siegel Neurobiology Research, VA GLAHS Sepulveda, UCLA Department of Psychiatry, David Geffen School of Medicine, Los Angeles, California, U.S.A.

Michael H. Silber Department of Neurology and Sleep Disorders Center, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

Karl E. Sonka Department of Neurology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Teresa L. Steininger Biosciences Division, SRI International, Menlo Park, California, U.S.A.

J. Gregor Sutcliffe Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, U.S.A.

Chisa Suzuki Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, U.S.A.

Mariana Szklo-Coxe University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

Mehdi Tafti Center for Integrative Genomics, University of Lausanne, Lausanne-Dorigny, Switzerland

Thomas C. Thannickal Neurobiology Research, VA GLAHS Sepulveda, UCLA Department of Psychiatry, David Geffen School of Medicine, Los Angeles, California, U.S.A.

Michael Thorpy Department of Neurology, Sleep-Wake Disorders Center, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, U.S.A.

Katsushi Tokunaga Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

J. Gert Van Dijk Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

Shirley Whittom Centre d'Étude du Sommeil, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada

Jon T. Willie Department of Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Raphaëlle Winsky-Sommerer Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, U.S.A.

Xinmin S. Xie Biosciences Division, SRI International, Menlo Park, California, U.S.A.

Masashi Yanagisawa Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A. and ERATO Yanagisawa Orphan Receptor Project, Japan Science and Technology Agency, Tokyo, Japan

Terry Young University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

Jamie M. Zeitzer Stanford University School of Medicine, Stanford, California, U.S.A.

Contents

Introduction Claude Lenfant . . . . . *iii*
Preface . . . . . *v*
Contributors . . . . . *vii*

1. Historical Aspects of Narcolepsy *1*
William C. Dement
I. Pre-REM Years *2*
II. The Discovery of REM Sleep *2*
III. Sleep Onset REM Periods *2*
IV. Efforts to Establish Prevalence *3*
V. Discovery of Canine Narcolepsy *4*
References *4*

2. English Translations of the First Clinical Reports on Narcolepsy by Gélineau and on Cataplexy by Westphal in the Late 19th Century, with Commentary *7*
Carlos H. Schenck, Claudio L. Bassetti, Isabelle Arnulf, and Emmanuel Mignot
I. Gélineau's Description of Narcolepsy *8*
II. Westphal's Description of Narcolepsy–Cataplexy *9*
III. The Authors *10*
IV. Further Comments on Westphal's and Gélineau's Descriptions of Narcolepsy *12*
References *14*
V. The English Translations *15*

3. Historical Aspects of the Treatment for Narcolepsy *25*
Yasuo Hishikawa and Tetsuo Shimizu
I. Introduction *25*
II. Pharmacologic Treatment *25*
III. Psychosocial Counseling and Behavioral Modification *28*
IV. Conclusions *29*
References *29*

4. Narcolepsy and Hypersomnia: Immunogenetic Aspects of Narcolepsy—Past, Present, and Future *31*
Yutaka Honda, Minae Kawashima, and Katsushi Tokunaga
I. Introduction *31*
II. The Diagnosis of Narcolepsy and HLA DR2/DRB1*1501 Frequency in Japan *32*
III. HLA Study of Japanese Families with Multiple Narcoleptic Patients *33*
IV. An Independent Association of Tumor Necrosis Factor-Alpha (TNF-Alpha) Promoter Gene Polymorphism in Narcolepsy *36*
V. Twin Studies of Narcolepsy *36*
VI. Decreased Hypocretin Levels in the Cerebrospinal Fluid of Narcoleptic Patients *37*
VII. A Genome-Wide Search for the Susceptibility Genes of Narcolepsy *38*
VIII. Postmortem Brain Studies in Narcolepsy *40*
References *41*

5. The Hypocretins: Discovery and Emerging Role as Integrators of Physiological Signals *43*
Luis de Lecea, Raphaëlle Winsky-Sommerer, Covadonga Pañeda,o Chisa Suzuki, and J. Gregor Sutcliffe
I. Hypocretin Discovery *43*
II. Hypocretin Neurons Integrate Metabolic Information *45*
III. Hypocretins Set the Arousal Threshold *45*
IV. Hypocretins, Stress, and Addiction *46*
V. Conclusion *47*
References *47*

6. Cataplexy *49*
Christian Guilleminault, Ji Hyun Lee, and Viola Arias
I. Introduction *49*
II. Clinical Characteristics *49*
III. The Secondary Cataplexy *52*
IV. The Neurophysiology of Cataplexy *53*
V. Animal Studies *55*

VI. Pharmacological Investigations *55*
VII. Cataplexy and HLA Typing *56*
VIII. Familial Aspect of Cataplexy and HLA *57*
IX. HLA and Hypocretin/Orexin *57*
X. The Canine Model of Cataplexy and EDS *58*
XI. Conclusion *59*
References *59*

7. Effect of Age on Narcolepsy *63*
Yves Dauvilliers, Jacques Montplaisir, and Michel Billiard
I. Biphasic Distribution of Age at Onset *63*
II. Effects of Age on Multiple Sleep Latency Test *64*
References *66*

8. Narcolepsy in Children and Adolescents *67*
Soňa Nevšímalová
I. Clinical Presentation of Narcolepsy in Children: An Overview *67*
II. A Case of Hypocretin-Deficient Narcolepsy Due to a Mutation in the Hypocretin Gene *70*
III. Secondary Cases of Narcolepsy in Children *70*
IV. Diagnosing Narcolepsy in Children *71*
V. Treatment Issues in the Pediatric Population *73*
VI. Conclusion *74*
References *74*

9. Idiopathic Hypersomnia *77*
Michel Billiard and Yves Dauvilliers
I. Historical Background *77*
II. Epidemiology *78*
III. Clinical Features *78*
IV. Laboratory Tests *79*
V. Frontiers *81*
VI. Differential Diagnosis *81*
VII. Pathophysiology *83*
VIII. Treatment *84*
IX. Conclusion *85*
References *85*

10. Kleine-Levin Syndrome and Other Recurrent Hypersomnias *89*
Claudio Bassetti, Michel Billiard, Emmanuel Mignot, and Isabelle Arnulf
I. Epidemiology *89*
II. Clinical Features *91*

III. Pathophysiology 92
IV. Etiology 92
V. Treatment 94
References 94

11. Spectrum of Narcolepsy 97
Claudio L. Bassetti
I. Introduction 97
II. Narcolepsy Without Cataplexy 98
III. Other Forms of Monosymptomatic Narcolepsy/Isolated Cataplexy 100
IV. Familial Narcolepsy/Narcolepsy in Twins 100
V. HLA-Negative Narcolepsy and Narcolepsy with Normal CSF Hypocretin-1 Levels 101
VI. Symptomatic Narcolepsy 102
VII. "Psychiatric" Narcolepsy 103
VIII. Summary and Conclusions 104
References 105

12. Sleep Paralysis, State Transition Disruptions, and Narcolepsy . . . 109
James Allan Cheyne
I. A Brief Neurophenomenology of Sleep Paralysis Experiences 109
II. Narcolepsy, State-Transition Disruption, and Severity of Sleep Paralysis 111
III. Conclusions 116
References 116

13. Epidemiology of Narcolepsy: Development of the Ullanlinna Narcolepsy Scale 119
Christer Hublin
I. Prologue 119
II. The Finnish Twin Cohort and the Research Group 119
III. Where Does the Name Ullanlinna Come From? 120
IV. The Development of the Ullanlinna Narcolepsy Scale (UNS) 120
V. The Finnish Prevalence Study 120
VI. Conclusions 122
VII. Epilogue 122
References 123

14. Epidemiology of Narcolepsy 125
Maurice M. Ohayon
I. Prevalence in the United States 125
II. Prevalence in Europe 125

III. Prevalence in Asia *129*
IV. Prevalence in Middle East *130*
V. Conclusions *130*
References *131*

15. Automatic Behavior, Sleep Paralysis, Hypnagogic Hallucinations, Cataplexy: Narcolepsy Spectrum and Alternate Etiologies *133*
Mariana Szklo-Coxe, Terry Young, Laurel Finn, and Emmanuel Mignot
I. Introduction *133*
II. Methods and Materials *134*
III. Analyses *136*
IV. Results *137*
V. Discussion *144*
VI. Conclusions *146*
VII. Funding *147*
References *148*

16. Neurophysiology of Cataplexy and Cataplexy-Like Phenomena *151*
Sebastiaan Overeem, Gert Jan Lammers, Bastiaan R. Bloem, and J. Gert Van Dijk
I. Introduction *151*
II. Clinical Aspects *152*
III. Neurobiology of Cataplexy *153*
IV. Neurophysiological Investigations During Cataplexy *155*
V. Neurophysiological Studies Between Cataplectic Attacks *158*
VI. Cataplexy-Like Phenomena *159*
VII. Neurophysiological Methods as a Diagnostic Aid? *160*
VIII. Future Perspectives *161*
References *162*

17. Abnormal Motor Activity During Sleep in Narcolepsy *165*
Jacques Montplaisir, Shirley Whittom, Sylvie Rompré, Thien Dang-vu, and Yves Dauvilliers
I. Periodic Leg Movements in Sleep (PLMS) *165*
II. REM Sleep Behavior Disorder (RBD) *168*
References *169*

18. Circadian and Ultradian Aspects in Narcolepsy *171*
Franco Ferrillo and Lino Nobili
I. Circadian Aspects *171*
II. Circasemidian Aspects *171*
III. Ultradian Aspects *172*

IV. Homeostatic Aspects 172
V. The Bed-Rest Protocol 172
VI. An Attempt to Simulate Features of Sleep in Narcolepsy 174
VII. Comments 180
References 183

19. Homeostatic Sleep Regulation in Narcolepsy 187
Ramin Khatami, Peter Achermann, Hans-Peter Landolt, and Claudio L. Bassetti
I. Introduction 187
II. NREM-Sleep Homeostasis in Human Narcolepsy 188
III. REM-Sleep Homeostasis in Human Narcolepsy 189
IV. Discussion 190
V. Conclusions 190
References 191

20. Daytime Variations in Alertness/Drowsiness and Vigilance in Narcolepsy/Cataplexy 193
Roger J. Broughton
I. Physiological Measures of Daytime Alertness/Drowsiness Variations in Narcolepsy 193
II. Do Qualitatively Different States of Sleepiness Exist? 197
III. Variations in Alertness/Drowsiness and Performance Vigilance in Narcolepsy 200
IV. What Is the Main Cause of the Marked Daytime Drowsiness Characterizing Narcolepsy? 203
References 207

21. Molecular Characterization of Hypocretin/Orexin and Melanin Concentrating Hormone Neurons: Relevance to Narcolepsy 211
Christelle Peyron
I. Hypocretins/Orexins 211
II. Melanin Concentrating Hormone 212
III. Co-Expression Data 213
IV. Conclusion 215
References 216

22. Nocturnal Polysomnography, Multiple Sleep Latency Test and Maintenance of Wakefulness Test in Narcolepsy 219
Merrill M. Mitler
I. Summary 219
II. Introduction 219

III. Nocturnal Polysomnography *221*
IV. The Multiple Sleep Latency Test *222*
V. Sleep Onset REM Periods *224*
VI. The Maintenance of Wakefulness Test *224*
VII. Conclusion *225*
References *228*

23. Canine Narcolepsy: History and Pathophysiology *231*
Emmanuel Mignot
I. Early History *231*
II. Early Clinical, Pharmacological and Electrophysiological Characterization of the Canine Narcolepsy Model *234*
III. Neurochemical Studies in Canine Narcolepsy *235*
IV. Further Pharmacological Studies in Canine Narcolepsy *236*
V. Local Injections and In Vivo Dialysis Studies *237*
VI. Positional Cloning Studies in Canine Narcolepsy *238*
VII. Hypocretin Deficiency Without DLA-DQB1 Association in Sporadic Narcolepsy Cases *239*
VIII. Perspectives *239*
References *240*

24. Vigilance Tests in Narcolepsy *243*
Johannes Mathis and Christian W. Hess
I. Pathophysiology and Etiology of EDS *243*
II. Questionnaires *244*
III. Multiple Sleep Latency Test (MSLT) *246*
IV. Limitations of the MSLT *247*
V. Maintenance of Wakefulness Test (MWT) *248*
VI. Reaction Time Tests *249*
VII. Pupillography *250*
VIII. Driving Simulators *250*
IX. Continuous Ambulatory EEG Monitoring *251*
X. Complex Event Related Potentials *251*
XI. Actigraphy *251*
XII. Assessment of Sleepiness in Children *253*
XIII. Summary and Perspectives *253*
References *253*

25. Lessons from Sleepy Mice: Narcolepsy-Cataplexy and the Orexin Neuropeptide System *257*
Jon T. Willie and Masashi Yanagisawa
I. Introduction *257*
II. Molecular Genetic Analysis of Narcolepsy-Cataplexy in Mice *258*

III. Probing the Nature of Sleepiness in Murine Narcolepsy-Cataplexy *269*
IV. Orexin to the "Rescue": Therapy for Narcolepsy-Cataplexy *272*
V. Conclusion: A Molecular Genetic Model of the Narcolepsy-Cataplexy Phenotype *274*
VI. Summary *276*
References *276*

26. Hypocretin-1 Studies in Cerebrospinal Fluid: European Experience ***279***
Christian R. Baumann, Claudio L. Bassetti, Sebastiaan Overeem, and Gert Jan Lammers
I. Introduction *279*
II. Determination of CSF Hypocretin-1 Levels *279*
III. CSF Hypocretin-1 in Narcolepsy and Other Disorders *280*
IV. Summary and Conclusions *284*
References *285*

27. CSF Hypocretin-1/Orexin-A in Narcolepsy: Technical Aspects and Clinical Experience in the United States ***287***
Emmanuel Mignot
I. CSF Hypocretin-1: Technical Aspects *287*
II. CSF Hypocretin-1: Stability of CSF Measurements, Effects of Drugs, Circadian Time, and Other Manipulations *289*
III. Effects of Other Sleep, Neurologic, and Psychiatric Conditions *291*
IV. CSF Hypocretin-1 in Narcolepsy-Cataplexy *292*
V. CSF Hypocretin-1 in Narcolepsy Without Cataplexy *293*
VI. Pathophysiological Models of the Narcolepsy Spectrum: Continuum or Heterogeneity *293*
VII. CSF Hypocretin-1 in Secondary Hypersomnia: Fact or Fiction *295*
VIII. Diagnostic Utility of CSF Hypocretin-1 .... *295*
IX. Perspective for a Plasma Assay *297*
X. Conclusion *297*
References *297*

28. Hypocretin Pathology in Human Narcolepsy ***301***
Thomas C. Thannickal and Jerome M. Siegel
I. Introduction *301*
References *304*

29. Symptomatic Narcolepsy with Cataplexy and Without Cataplexy or Hypersomnia, with and Without Hypocretin (Orexin) Deficiency ***307***
T. Kanbayashi, M. Abe, Yasuo Hishikawa, Tetsuo Shimizu, and Seiji Nishino
I. Introduction *307*
II. Hypocretin Status in Various Neurological Conditions *315*
III. Hypocretin Status in Hypersomnia in Various Neurological Conditions *319*
IV. Conclusion *328*
References *330*

30. Poststroke and Posttraumatic Hypersomnia ***335***
Claudio L. Bassetti and Christian R. Baumann
I. Introduction *335*
II. Epidemiology *335*
III. Clinical Features *336*
IV. Pathophysiology *338*
V. Diagnosis *341*
VI. Treatment *342*
VII. Conclusions *343*
References *343*

31. Potential Mechanisms of the Wake-Promoting Action of Hypocretin/Orexin ***347***
Michael Mühlethaler
I. Introduction *347*
II. Conclusion *349*
References *353*

32. Normal Role of Hypocretin/Orexin ***355***
Jerome M. Siegel
I. Introduction *355*
II. Conclusion *357*
References *358*

33. Role of Hypocretin/Orexin in the Neurobiology of Sleep and Alertness ***359***
Jamie M. Zeitzer and Emmanuel Mignot
I. Hypocretin Neurobiology *359*
II. Effects of Exogenously Applied Hypocretins *359*
III. Downstream Mediators of Hypocretin Effects on Wakefulness *360*
IV. CSF Hypocretin-1 as a Useful Proxy of Hypocretin Release *361*

V. Diurnal Fluctuation and Circadian Regulation of Hypocretin Release 363
VI. Variable Effects of Stress, Locomotion and Manipulation of Food Intake on Hypocretin Release 363
VII. Activation of Hypocretin Release by Sleep Deprivation 367
VIII. Sleep Stage Regulation of Hypocretin Activity 368
IX. Functional Relevance to the Narcolepsy Phenotype 369
X. Perspectives 371
XI. Summary 371
References 371

34. Hypocretin, $GABA_B$ Receptors and Sleep 375
Thomas S. Kilduff, Stephen R. Morairty, Teresa L. Steininger, and Xinmin S. Xie
I. Introduction 375
II. Narcolepsy, Gamma-Hydroxybutyrate, and the Hypocretin System 376
III. Perspective and Future Directions 382
References 383

35. The Activity Profile of Hypocretin Neurons in the Freely Moving Rat 387
Boris Y. Mileykovskiy, Lyudmila I. Kiyashchenko, and Jerome M. Siegel
I. Introduction 387
II. Electrophysiological Identification of Hcrt Neurons 387
III. Hcrt Neuronal Activity During Sleep 388
IV. Hcrt Neuronal Activity and Feeding Behavior 391
V. Hcrt Neuronal Activity and Motor Activity 391
VI. Hcrt Neuronal Activity and Cataplexy 392
VII. Hcrt Neuronal Activity and Stress 392
VIII. Hcrt Neuronal Activity and Psychotropic Drugs 394
IX. Conclusion 394
References 395

36. Input and Output of Orexin/Hypocretin Neurons: Link Between Arousal Pathways and Feeding Behavior 399
Takeshi Sakurai
I. Introduction 399
II. Efferents of Orexin Neurons 399
III. Afferents of Orexin Neurons 404
IV. Summary 407
V. Conclusion 408
References 408

37. Human Leukocyte Antigen and Narcolepsy: Present Status and Relationship with Familial History and Hypocretin Deficiency ***411***
Ling Lin and Emmanuel Mignot
I. Introduction *411*
II. HLA Gene: Structure and Function *413*
III. HLA-DQB1*0602 Susceptibility and Narcolepsy: A Complex Association *414*
IV. HLA Alleles in Typical, Atypical Narcolepsy, and Idiopathic Hypersomnia *419*
V. HLA Association and Hypocretin-1 Deficiency *419*
VI. Recent Autoimmune Studies *422*
VII. Perspectives *423*
References *424*

38. Non-HLA Genes in Narcolepsy ***427***
Mehdi Tafti
I. Genetic Susceptibility to Narcolepsy in Families and Twins *428*
II. Candidate Gene Approach in Narcolepsy *429*
III. Linkage Studies in Narcolepsy *431*
IV. Conclusions *432*
References *432*

39. Mutation Screening of the Hypocretin System Genes in Narcolepsy ***435***
Juliette Faraco and Emmanuel Mignot
I. Introduction *435*
II. Studies Focused on DQ-Negative Subjects, Multiplex Families, and Proband-Parent Trios *436*
III. A Hypocretin Mutation in a Single Case of Narcolepsy *437*
IV. Hypocretin Screening in DQ-Positive Subjects *437*
V. Conclusion *440*
References *441*

40. Environmental Factors in Narcolepsy ***443***
Michel Billiard and Cecilia Orellana
I. Introduction *443*
II. Focused Studies *446*
III. Mechanisms *448*
IV. For the Future *449*
V. Conclusion *449*
References *449*

41. Autoimmune Studies in Narcolepsy *451*
Michael H. Silber, John L. Black, Lois E. Krahn, and Paul A. Fredrickson
I. Studies of Humoral Immunity (Excluding the Hypocretin System) *451*
II. Studies of Cell Mediated Immunity and Cytokine Levels *453*
III. Studies of the Hypocretin System *453*
IV. Narcolepsy in Other Autoimmune Diseases *455*
V. Pathology Studies *455*
VI. Future Directions *455*
References *456*

42. Metabolic Abnormalities in Human Narcolepsy *459*
Thomas Pollmächer, Pierre Beitinger, and Andreas Schuld
I. Introduction *459*
II. Body Weight and Body Composition in Human Narcolepsy *459*
III. Appetite in Human Narcolepsy *460*
IV. Metabolic Abnormalities in Human Narcolepsy *461*
V. Causes of Alterations in Body Weight, Appetite, and Metabolism in Narcolepsy *461*
VI. Conclusions and Perspectives *463*
References *464*

43. Hormonal Abnormalities in Hypocretin/Orexin Deficient Human Narcolepsy *467*
Gert Jan Lammers, Sebastiaan Overeem, and Hanno Pijl
I. Introduction *467*
II. Historical Data *467*
III. Neuroendocrine Studies in Hypocretin Deficient Narcolepsy *468*
IV. Results *469*
V. Discussion *470*
References *471*

44. Psychosocial Impact of Narcolepsy *473*
Adrian M. Siegel and Roger J. Broughton
I. Narcolepsy *473*
II. Learning Deficits (At School, College, University) *473*
III. Difficulties at Work *474*
IV. Difficulties at Home and with Leisure *475*
V. Road and Work Accidents *475*
VI. Problems in Relationships and Sexual Activities *476*
VII. Improving the Psychosocial Impact of Narcolepsy *476*
References *476*

45. Narcolepsy and Driving ***479***
Jean Krieger and Christine Erhardt
I. Introduction *479*
II. Evidence from Patient Reports *479*
III. Evaluation of Driving Performance *480*
IV. Expert Reports *481*
V. Treatment *482*
VI. Regulations Concerning Driving and Narcolepsy *482*
VII. Conclusions *483*
References *484*

46. Comorbidity in Narcolepsy ***485***
Geert Mayer, Thomas Penzel, and Karl Kesper
I. Introduction *485*
II. Comorbidity and the German Narcolepsy Register *486*
III. Comorbidity *488*
IV. Parasomnias *488*
V. Intrinsic Sleep Disorders *492*
VI. Internistic Diseases *492*
VII. Neurological Diseases *493*
VIII. Psychiatric Disorders *493*
IX. Conclusion *494*
References *494*

47. The Behavioral Management of Narcolepsy ***497***
Roger J. Broughton and Brian J. Murray
I. Introduction *497*
II. Night-Time Symptoms *498*
III. Daytime Symptoms *499*
IV. Psychiatric Problems *504*
V. Obesity and Nutrition *505*
VI. Psychosocial Impact and Behavioral Interventions *506*
VII. Coexistent Sleep Disorders *507*
VIII. Can the Risk of Developing Narcolepsy be Minimized by Behavioral Intervention? *507*
IX. Patient and Family Education and Counseling *508*
X. Medical Education *508*
References *509*

48. Pharmacology of CNS Stimulants ***513***
Seiji Nishino and Emmanuel Mignot
I. Introduction *513*
II. Amphetamines and Amphetamine-Like Compounds *513*
III. Molecular Targets of Amphetamine Action *516*
IV. Dopaminergic Neurotransmission and EEG Arousal *518*

V. Anatomical Substrates of Dopaminergic Effects *521*
VI. Clinical Pharmacology of Amphetamine and Amphetamine-Like Compounds *521*
VII. Non-Amphetamine Stimulants *522*
VIII. Future Stimulant Treatments *527*
IX. Conclusion *528*
References *529*

49. Anticataplectic Medications *531*
Seiji Nishino
I. Introduction *531*
II. Pharmacology of Antidepressants *531*
III. Canine Narcolepsy Model for Understanding the Pharmacological Control of Cataplexy *533*
IV. Pharmacological Treatment of Cataplexy in Humans *538*
V. Treatment of Sleep Paralysis and Hypnagogic Hallucinations *543*
VI. Hypocretin Agonists as Potential Therapeutic Agents *543*
VII. Conclusion *544*
References *545*

50. Modafinil: Mechanisms of Action *547*
Thomas E. Scammell
I. Introduction *547*
II. Neurobiology of Wakefulness *547*
III. Amphetamines *549*
IV. Modafinil's Mechanisms of Action *550*
V. Modafinil Differs from Amphetamine in Many Ways *554*
VI. Conclusions *555*
References *556*

51. Modafinil: The European Experience *561*
Michel Billiard, Agnes Nicolet, Yves Dauvilliers, and Bertrand Carlander
I. Discovery and Development of Modafinil *561*
II. First Clinical Publications *563*
III. Potential Brain Arousal Targets for Amphetamine, Methylphenidate, and Modafinil-Induced Wakefulness, Evidenced by c-*fos* Immunocytochemistry in the Cat and the Rat *564*
IV. Functional Polymorphism of the COMT Gene as a Critical Factor in the Response to Modafinil *565*
V. Long-Term Efficacy and Safety of Modafinil for the Treatment of Excessive Daytime Sleepiness in Narcoleptic Subjects *566*
VI. Conclusion *567*
References *567*

52. Modafinil: The U.S. Experience ***571***
Michael Thorpy
I. The Introduction of Modafinil in the United States *571*
II. The U.S. Clinical Trials of Modafinil in Narcolepsy *571*
III. Expanding the Indications for Modafinil *575*
IV. U.S. Safety and Long-Term Efficacy Data with Modafinil *576*
V. Future Directions for Modafinil Research *578*
VI. Summary *579*
References *580*

53. Molecular and Cellular Actions of γ-Hydroxybutyric Acid: Possible Mechanisms Underlying GHB Efficacy in Narcolepsy ***583***
Xinmin S. Xie, Daniel Pardi, and Jed Black
I. Introduction *583*
II. Basic GHB Neurochemistry *585*
III. Cellular Actions of GHB *588*
IV. GHB as a Neuromodulator *596*
V. Relevance to Narcolepsy *603*
VI. Conclusions *609*
References *610*

54. Sodium Oxybate for the Treatment of Narcolepsy ***621***
Jed Black
I. Overview *621*
II. Introduction *621*
III. Initial Exploration in Narcolepsy *622*
IV. Further Open-Label Work *622*
V. Initial Long-Term Experience *623*
VI. First Placebo-Controlled Trials *623*
VII. Formal Development of Sodium Oxybate for the Treatment of Narcolepsy *625*
VIII. Safety and Tolerability in Narcolepsy *634*
IX. Pharmacokinetics, Drug Interactions and Dosing Considerations *635*
X. Illicit Use and Abuse Potential *637*
XI. Conclusion *637*
References *637*

55. Perspectives for New Treatments: An Update ***641***
Emmanuel Mignot and Seiji Nishino
I. Improving Current Therapeutic Strategies Using Novel Antidepressants, Stimulants, and Hypnotics *641*
II. Non-Catecholaminergic Stimulants *644*
III. Hypocretin Peptide Supplementation *645*

IV. Immunomodulation as a Preventive Treatment for Narcolepsy 650
References 652

56. Diagnostic Criteria for Syndromes of Excessive Daytime Sleepiness 655
Emmanuel Mignot, Michel Billiard, and Claudio L. Bassetti
I. Introduction 655
II. On the Use and the Misuses of the Multiple Sleep Latency Test (MSLT) 657
III. Narcolepsy with and Without Cataplexy 657
IV. Idiopathic Hypersomnia with and Without Long Sleep Time 659
V. Need for Future Studies 660
References 660

Appendix A. Guidelines for the Appropriate Use of CSF Measurements to Diagnose Narcolepsy 663
Ling Lin, Claudio L. Bassetti, Gert Jan Lammers, Seiji Nishino, Michael H. Silber, T. Kanbayashi, Jamie M. Zeitzer, Michel Billiard, Sebastiaan Overeem, Yves Dauvilliers, Laurence K. Oliver, Geert Mayer, and Emmanuel Mignot
I. Introduction 663
II. Hcrt-1 Radioimmunoassay (RIA): Principles and Pitfalls 663
III. Lumbar Puncture and Stability of CSF for Hcrt-1 Assays 665
IV. Hcrt-1 Radioimmunoassay (RIA) Protocol 666
V. Suggested Reading 670

Appendix B. Treatment Guidelines 671
Michel Billiard, Claudio L. Bassetti, Jed Black, Roger J. Broughton, Yves Dauvilliers, Yutaka Honda, T. Kanbayashi, Gert Jan Lammers, Geert Mayer, Emmanuel Mignot, Jacques Montplaisir, Thomas Pollmächer, Thomas E. Scammell, Michael H. Silber, Karl E. Sonka, and Michael Thorpy
I. Introduction 671
II. Excessive Daytime Sleepiness and Irresistible Episodes of Sleep 671
III. Cataplexy and Auxiliary Symptoms, Hallucinations, and Sleep Paralysis 672
IV. General Recommendations 674
V. Future Treatments 674
VI. Conclusion 675
References 675

Index 677

1
Historical Aspects of Narcolepsy

WILLIAM C. DEMENT
Stanford University School of Medicine, Palo Alto, California, U.S.A.

It is the strong opinion of this author that research on narcolepsy and the diagnosis and treatment of patients afflicted with narcolepsy have a value not only for narcolepsy patients but also a value beyond a sole concern about the illness. The following brief history will support this opinion.

We know that insomnia symptoms and other types of disturbances such as sleepwalking and night terrors have been a problem for humans almost certainly since the dawn of history. They are mentioned in the writings of Aristotle and others. However, the first clear description of a specific sleep pathology was published by Jean-Baptiste Edouard Gelineau (1828–1906) in 1880. Several patients who surely had narcolepsy had been described previously by others, but Gelineau realized that the characteristic symptoms almost always clustered and bestowed upon them the name "narcolepsy." The Gelineau report (1), which appeared in the Gazette des Hospitals, describes how emotions influence the onset of sleep attacks and how some attacks were quite literally falling down. Gelineau also felt that narcolepsy should be regarded as an autonomous disease and should not be confused with epileptic seizures.

Narcolepsy research has revealed additional characteristics of the illness beyond the classical tetrad. Nonetheless, the four features that comprised the syndrome in the original description are as follows (2):

1. Excessive and persistent somnolence: daytime sleepiness at inappropriate times, or sleep attacks, sudden urges to sleep, without regard to either the amount or the quality of prior nighttime sleep.
2. Cataplexy: episodes of partial or general muscular weakness induced by emotions, such as laughter, anger, or surprise.
3. Hypnagogic hallucinations: vivid, realistic, and sometimes frightening auditory or visual perceptions which occur at sleep onset.
4. Sleep paralysis: episodes of temporary inability to move or speak, which occur while falling asleep or awakening.

There are other consistent features of narcolepsy as well as exciting new research on neurotransmitter systems in the brain which will be described throughout this book.

I. Pre-REM Years

The illness of narcolepsy, particularly attacks of cataplexy, though not highly prevalent, seems sufficiently interesting that it should not have been so generally ignored. On the other hand, narcolepsy did eventually capture the interest of a few individuals. A large case series was accumulated at the Mayo Clinic, first reported by Daniels (3) and subsequently by Yoss and Daly (4). Bedrich Roth in Czechoslovakia also focused on narcolepsy and accumulated a large number of cases. He published an early monograph on narcolepsy and the second edition was finally translated into English in 1980 (5). In spite of the clarity of the Gelineau description of a syndrome, the inability to conceive a single mechanism that could parsimoniously account for complete muscular atonia triggered by strong emotion on the one hand, and relentless sleepiness on the other, fostered great misunderstanding and misinterpretation. For example, the huge numbers of individuals who survived the encephalitis epidemic of 1917 were labeled narcoleptics. With a turn-of-the-century Freudian emphasis on hysteria and conversion, the impression grew that the cataplectic attacks often triggered by anger were a hysterical defense against aggressive behavior. Many health professionals, including the very eminent British neurologist, Sir Russell Brain, continued to believe that narcolepsy was a form of epilepsy. Finally, Yoss and Daly diagnosed "independent narcolepsy" when patients complained only of sleepiness.

II. The Discovery of REM Sleep

It would probably be quite accurate to say that the narcolepsy syndrome was destined to remain a mystery unless and until REM sleep with its unique physiology was discovered and thoroughly described. As recently as 1952, rapid eye movements during sleep were not known to exist. Even after the first observations (6,7,8), REM periods were considered a form of light sleep. Moreover, the initial interest in REM sleep was mainly its relation to vivid dreaming demonstrated by the very high percentage of detailed dream recall when volunteers were awakened from REM periods.

The discovery of REM sleep in cats (9) with the associated EMG suppression, and Michel Jouvet's elegant studies of muscular atonia and its brain stem substrates also in cats (10,11) clinched the concept of the duality of sleep. According to this concept, REM periods constituted an independent state of being associated with muscular paralysis, vivid hallucinatory dreaming and activated EEG.

III. Sleep Onset REM Periods

The occurrence of sleep onset REM periods in a patient with narcolepsy was reported by Vogel in 1960 (12). A larger group of nine patients was reported by Rechtschaffen et al. a few years later (13). The abnormal occurrence of REM sleep at the onset of sleep was the obvious explanation for the occurrence of hypnogogic hallucinations and sleep paralysis in narcoleptic patients. Several studies in normal animals showed that REM sleep was associated with an active inhibition of alpha and gamma spinal motor neurons (14). This inhibitory process was found to be initiated by centers in the pontine tegmentum.

The evidence of a REM sleep abnormality in narcoleptic patients in terms of sleep onset REM periods led to the conclusion that cataplexy was the initiation of REM atonia in the waking state triggered by strong emotion. This was confirmed by recording one or two daytime naps in a large number of patients complaining of sleepiness (15). Those who also complained of cataplexy had sleep onset REM periods and those who did not complain of cataplexy also did not have SOREMPs. Subsequently, it was realized that most of the sleepy patients *without* cataplexy were suffering from obstructive sleep apnea.

The large number of patients accumulated in the sleep onset REM periods/cataplexy study were identified and recruited by placing a small advertisement in the *San Francisco Chronicle* daily newspaper. As these individuals came forward, it was found that none had a previous diagnosis of narcolepsy and, of course, none had been properly treated. The Stanford group was thus in the position of being responsible for the clinical management of several hundred patients with narcolepsy. In order to make this daunting task practical and feasible, a special narcolepsy clinic was launched. The clinic soon failed financially because patients generally were unable to pay for the diagnostic testing to demonstrate sleep onset REM periods in addition to the office visits.

This first experience of clinic dedicated to the diagnosis and treatment of one sleep disorder was the inspiration for a renewed effort to establish a clinic and the formal launch of the world's first full-service sleep disorders center diagnosing insomnia, sleep apnea, narcolepsy and other sleep disorders at Stanford in the summer of 1970. One may speculate that if the experiences of the narcolepsy clinic had not taken place and had not been a satisfying mode of clinical practice, the Stanford Sleep Center would never have been launched.

IV. Efforts to Establish Prevalence

The majority of the patients referred to the Stanford Sleep Disorders Clinic were thought to have narcolepsy by their referring physicians. This would suggest a much higher prevalence in society than previously thought. In view of this, we decided to try to establish a reliable population prevalence for narcolepsy in the United States. The first effort involved newspaper advertising. Very large displays were placed in three bay area newspapers (total circulation 1,200,000) requesting persons with certain characteristics to respond. The study had controls and a rationale for arriving at the final result which was a "conservative estimate of the number of narcoleptics (sleepiness plus cataplexy) in the USA is 100,000 (.05%)." We tried to publish our results and received a great deal of critical review from epidemiologists, along with a series of rejections. Since we eventually physically examined and tested the survey respondents, we were fairly confident of our results. However, because of the continuing skepticism, another study was carried out in the Los Angeles area utilizing television broadcasting with a film depicting sleep attacks and cataplexy. The second study allowed a conservative conclusion that "there are 130,000 (.067%) Americans who suffered from narcolepsy." Studies since this time have raised the figure to about 200,000. We were also unable to publish the results of the second study. Both are reasonably thoroughly described in the abstracts (16,17).

V. Discovery of Canine Narcolepsy

The Stanford staff kept wider records of cataplectic attacks for educational purposes. These were exhibited at an American Medical Association convention in San Francisco in 1972. Seeing the film of human cataplexy, one neurologist informed the Stanford group that a Doberman pinscher with the same behavior had been observed at the University of California, Davis, School of Veterinary Medicine. When the attending veterinarian was contacted, it was learned that he had sacrificed the dog because it suffered from "intractable, untreatable epilepsy." However, he had made a movie of the dog's "seizures" which he sent to Stanford. In the film, global muscular atonia occurred which closely resembled a cataplectic attack whenever the dog approached a bowl of food.

Subsequently, movies of human cataplexy made by the Stanford group, together with the movie of the UC Davis Doberman pinscher collapsing were shown at an American Association of Neurology meeting in Boston in 1973. A neurologist at this meeting reported that he was aware of a dog which showed these periodic collapses. The dog, a French poodle, was alive and well in Saskatoon, Saskatchewan, and the owners were persuaded to donate her to the Stanford Sleep Center. When the dog arrived she was quickly proven to have classical REM atonia/sleep onset REM periods and possibly to be excessively sleepy. The latter was more difficult to establish because excessive sleepiness is primarily a subjective report.

Reasoning that if one dog with narcolepsy existed there had to be others, I undertook a national search by giving lectures at every possible location of veterinary schools and animal care centers. My lectures included a meticulous description of canine narcolepsy and were accompanied by movies of canine narcolepsy. Our second narcoleptic dog was also a French poodle. From 1974–1975, we received a number of dogs from veterinarians around the United States. With considerable difficulty because of inexperience and inadequate facilities, we nonetheless bred male and female narcoleptic canines and whelped the puppies. Sometime in 1976, a litter from male and female narcoleptic Doberman pinschers appeared to be developing narcolepsy. However, the puppies became ill with viral encephalitis and all died. In 1977, a litter of five puppies were successfully delivered from narcoleptic Doberman pinscher parents and around 8 weeks of age, almost on the same day, all puppies developed obvious cataplexy. This litter received enormous media coverage. If we played with the puppies, all would have cataplectic attacks simultaneously. Ultimately a sizeable colony was established at Stanford, and with inbreeding, a heritable form of narcolepsy/cataplexy was established (18,19,20). This colony was maintained for more than 20 years until the narcolepsy gene was isolated by Emmanuel Mignot's group at Stanford in 1999 (21).

As the leading instigator of the early efforts, I am content that the considerable outlay of funds to house and feed a large colony of narcoleptic canines for twenty years has paid off, and paid off quite handsomely, I might add.

References

1. Gelineau J. De la narcolepsie. Gaz hop (Paris). 1880; 53:626–8.
2. Dement WC. The history of narcolepsy and other sleep disorders. J Hist Neurosci. 1993 Apr; 2(2):121–2.
3. Daniels L. Narcolepsy. Medicine (Baltimore). 1934; 12:1–122.

4. Yoss RE, Daly DD. Criteria for the diagnosis of the narcoleptic syndrome. Proc Staff Meet Mayo Clin. 1957; 32:320-8, 234.
5. Roth B. Narcolepsy and hypersomnia. Basel, Karger, 1980.
6. Aserinsky E, Kleitman N. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 1953; 118:273–4.
7. Dement WC. Dream recall and eye movements during sleep in schizophrenics and normals. J Nerv Ment Dis 1955; 122:263–269.
8. Dement WC, Kleitman N. Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. Electroencephalogr Clin Neurophysiol 1957; 9(4):673–90.
9. Dement WC. The occurrence of low voltage, fast, electroencephalogram patterns during behavioral sleep in the cat. Electroencephalogr Clin Neurophysiol 1958; 10(2):291–6.
10. Jouvet M, Michel F, Courjon J. Sur un stade d'activite electrique cerebrale rapide au cour du sommeil physiologique. Compt Rend Seanc Soc Biol 1959; 153:1024–8.
11. Jouvet M. Recherches sur les structures nerveuses et les mecanismes responsales des differentes phases du sommeil physiologique. Arch Ital Biol 1962; 100:125–206.
12. Vogel G. Studies in psychophysiology of dreams. III. The dream of narcolepsy. Arch Gen Psychiatry. 1960; 3:421–8.
13. Rechtschaffen A, Wolpert EA, Dement WC, Mitchell SA, Fisher C. Nocturnal sleep of narcoleptics. Electroencephalogr Clin Neurophysiol 1963; 15:599–609.
14. Pompeiano O. Mechanisms responsible for spinal inhibition during desynchronized sleep: Experimental study. In: Guilleminault C, Dement WC, Passouant P, eds. Advances in Sleep Research, Vol. 3, Narcolepsy. 1970; 411–49.
15. Dement W, Rechtshaffen A, Gulevich G. The nature of the narcoleptic sleep attack. Neurology 1966; 16(1):18–33.
16. Dement W, Zarcone V, Varner V, Hoddes E, Nassau S, Jacobs B, Brown J, McDonald A, Horan K. Glass R, Gonzales P, Friedman E, Phillips R. The prevalence of narcolepsy. Sleep Res 1972; 1:148.
17. Dement W, Carskadon M, Ley R. The prevalence of narcolepsy II. Sleep Res 1973; 2:147.
18. Mitler MM, Boysen BG, Campbell L, Dement WC, Narcolepsy-cataplexy in female dog. Exp Neurol 1974; 45(2):332–40.
19. Foutz AS, Mitler MM, Cavalli-Sforza LL, Dement WC. Genetic factors in canine narcolepsy. Sleep 1979; 1(4):413–21.
20. Baker TL, Foutz AS, McNerney V, Mitler MM, Dement WC. Canine model of narcolepsy genetic and developmental determinants. Exp Neurol 1982; 75(3):729–42.
21. Ling L, Faraco R, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–76.

2
English Translations of the First Clinical Reports on Narcolepsy by Gélineau and on Cataplexy by Westphal in the Late 19th Century, with Commentary

CARLOS H. SCHENCK
Department of Psychiatry, Minnesota Regional Sleep Disorders Center, Hennepin County Medical Center, University of Minnesota Medical School, Minneapolis, Minnesota, U.S.A.

CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

ISABELLE ARNULF
Fédération des Pathologies du Sommeil, Hôpital Pitié-Salpêtriére, Public Assistance, Hôpitaux de Paris, France

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

To our knowledge, there are no published English translations of the first clinical reports describing narcolepsy and cataplexy [in French, 1880, in two parts (1), and in German, 1877 (2)]. The first author of this chapter (CHS) previously had the Berlitz agency translate these two reports, and so this 2006 "state of the science" book on narcolepsy is a timely opportunity for presenting in English the original descriptions of narcolepsy and cataplexy. These historic documents richly describe recurrent, self-limited, sleep attacks and/or cataplectic attacks in two otherwise healthy people.

We have some preliminary comments concerning the translations. First, all punctuations and italics come from the original articles. Second, the article by Gélineau on narcolepsy was twice as long as the article by Westphal on cataplexy. Third, we edited the translations slightly in order to eliminate text that had no bearing on the description of narcolepsy or cataplexy. We eliminated 19 phrases or sentences and six paragraphs from the Gélineau reports, and three phrases or sentences and one paragraph from the Westphal report. We indicated the deletions with either ". . ." for the deleted phrases or sentences, or else "(paragraph deleted)." Fourth, the Berlitz translator of the Gélineau report made this comment: "The original French of this two-part article is written in an unusually loose style for late 19th century scientific reports. It is somewhat like a slightly-edited copying of hasty notes on a physician's note pad. Accordingly, it is difficult to render in smooth English; we have in many cases sacrificed esthetics of style for accuracy." Nevertheless, Passouant, who wrote about Gélineau for the narcolepsy centennial, mentioned that "Throughout his life, Gélineau wrote in a clear, alert, and

This chapter was adapted from a forthcoming paper to appear in the *Journal of Clinical Sleep Medicine*.

easy-to-read style (3)." It is evident that Gélineau astutely identified and accurately named *narcolepsy*, and he wrote an impressive set of descriptions on narcoleptic sleep attacks and their contexts, and he provided a detailed and carefully reasoned differential diagnosis and list of treatments.

We will now comment on certain other aspects of these two reports. (However, at this point the reader may wish to read the translations of the original texts contained in the final section of this chapter before returning to read our comments.)

I. Gélineau's Description of Narcolepsy

Dr. Jean Baptiste Edouard Gélineau (1828–1906) at the outset of his report attributed the initial description of narcolepsy to a Dr. Caffé who had published a case 18 years earlier, in 1862. However, our reading of Gélineau's quotes from Caffé's report would instead suggest the diagnosis of obstructive sleep apnea (OSA) as being more likely than narcolepsy. The case involved a 47-year-old man with "an irresistible and incessant propensity to sleep" that had forced him to resign from his job. He was not reported to have cataplexy, sleep paralysis or hypnagogic/hypnopompic hallucinations. However, he was reported to have "attitude detached; stupor; mental sluggishness; persistent stoutness; effect on overall health," and his face was "puffy." These descriptions are more indicative of OSA than of narcolepsy. Also, in consulting two different thesauruses concerning the word "stoutness," we found the following: One thesaurus had two physical meanings for this word (fatness, sturdiness), and of the 10 physical synonyms listed, nine were closely related to "fatness." The other thesaurus had two physical meanings for stoutness (size, strength), and of six physical synonyms listed, three pertained to fatness. In addition, the use of the word "persistent" to describe stoutness is much more likely to be a comment on an overweight or obesity status than of a strength or sturdiness status. For example, the phrase "a patient is persistently strong" would not be used, whereas "a patient is persistently overweight or obese" would be used. Therefore, Dr. Caffé presumably wished to convey an overweight or obese status concerning his patient when he used the word "stoutness." (One of the coauthors of this chapter—IA—reinforces this conclusion in regards to the word "fort" that describes a person being "overweight" distinctly more so than "strong," both in the 19th century and in the contemporary French language.) Although various treatments did not help Dr. Caffé's patient, a stay at a spa did improve his condition. Is it possible that he lost weight at the spa, which would have had a beneficial effect on his presumed OSA?

Gélineau presents a 38-year-old man with a two-year history of very frequent narcoleptic sleep attacks, totaling up to 200 attacks daily. This man could not speak with Dr. Gélineau for even 30 minutes without falling asleep, and constantly needed his 13-year-old son at his side to keep awakening him, so he could attend to his successful business. A wide array of intense emotional states played a prominent role in triggering his sleep attacks. The description of his initial visit with Dr. Gélineau is a dramatic example. In reading the entire report, a question could be raised as to whether this man—besides his "volatile temperament"—had histrionic personality traits that interacted with his narcolepsy. Gélineau briefly described cataplexy (which he termed falls or "astasia") and sleep paralysis in his patient, but did not comment on the presence of sleep-onset dreaming, dream disturbance, or hypnagogic/hypnopompic hallucinations. He mentioned that

his patient had "excellent night-time sleep, waking only once," which argues against the presence of either disruptive periodic limb movements of sleep or rapid eye movement (REM) sleep behavior disorder, conditions now known to be associated frequently with narcolepsy. Cataplexy was the initial manifestation of his narcolepsy.

Gélineau's patient was a member of the "mutual aid society," and his card bore the diagnosis, "*morbis sacer*," Latin for "sacred disease" in reference to epilepsy, which during antiquity had been considered a divine disorder. Gélineau's male patient reported that his infant child "was conceived in a moment when the illness came over him." Among the various explanations to account for this intriguing comment, the most likely would be either a hypnagogic hallucination or a vivid sleep-onset REM dream, which are common events with narcolepsy that may have accounted for an imagined sexual event. Another possibility is that this man indeed had coitus with his wife while awake that was immediately followed by a sleep attack, and in retrospect he incorrectly recalled the coitus to have occurred during the sleep attack. This patient received many unsuccessful treatments, including bromides, strychnine arsenate, curare, picrotoxin, apomorphine, phosphates, amyl nitrate vapors, hydrotherapy, electricity, and cauterization of the nape of his neck. Gélineau was thus led to comment, "as we both acknowledged that these successes were not in keeping with our mutual efforts, we lost contact, leaving to time and to nature the care of healing or improving this painful neurosis."

II. Westphal's Description of Narcolepsy–Cataplexy

Westphal had two cases that he presented at a Berlin Medical and Psychological Society meeting during 1877 that were then published in the *Archives of Psychiatry and Nervous Disorders* (for which he was an Editor). It is of note that he first chose to speak and write about "larvate epileptic attacks" before he described a patient with excessive daytime sleepiness and cataplectic attacks. Westphal emphasized in italics two aspects of his patient's clinical history: "*He did not lose consciousness during these attacks*," and "persistent *night-time sleeplessness* must be noted." Westphal clearly grasped that the cataplectic attacks involved loss of muscle tone without associated loss of consciousness, and his comment about sleeplessness indicated the presence of disrupted nocturnal sleep that is common (but not mandatory) in narcolepsy. In being the first investigator to describe narcolepsy with cataplexy, Westphal was also the first to describe familial narcolepsy, as the mother of his 36-year-old male patient had also suffered from longstanding sleep attacks and possibly cataplexy that was of milder severity than her son's cataplexy (although "she had been troubled by such attacks frequently earlier on"). Westphal also described repeated sleep attacks in his patient: "At times ... these attacks (viz. cataplexy) do cause the patient to fall asleep. The falling asleep appears, as it were, to be an extension or increase of the attack." The patient would also have sleep attacks in public while "still speaking" or while "strolling around quietly and aimlessly." These descriptions of sleep attacks and cataplectic attacks prove that Westphal correctly recognized and described narcolepsy with cataplexy before Gélineau, although he did not name these conditions, as did Gélineau for narcolepsy in 1880 and Henneberg for cataplexy in 1916 (4). It is noteworthy that only in 1902 a third author (Löwenfeld) confirmed Westphal's and Gelineau's suggestion that narcolepsy with cataplexy represents a "disease sui generis" (5).

III. The Authors

Let us be transported to 1878. The forceful unification of Germany by Prussia's Otto van Bismark has been completed after first defeating Austria and then the French armies during the short 1870 war against Napoleon the third. Germany is a strong but barely united country. France has lost the Alsace and the Lorraine and is a separate continent from Germany both culturally and linguistically. Psychoanalysis is not formally established. Sigmund Freud has not yet completed medical school, but there is growing interest in the unconscious and in psychological explanations for physical disorders. The pioneering work of Jean Martin Charcot's "Leçons sur les Maladies du Système Nerveux" has just been published, introducing the notion of hysteria. Neurology and Psychiatry are still in most countries all but one discipline.

Karl Friedrich Otto Westphal, born in 1833 in Berlin, is the son of a well-known and wealthy physician. After a European medical education that included studies in Germany, Switzerland, and France, he joined the smallpox clinic at the Berlin Charité hospital to rise to become full professor of Neurology and Psychiatry (Nervenkraknheiten) in 1865, where he trained a number of well-known physicians, including Arnold Pick and Carl Wernicke. His achievements are numerous and include the first descriptions of agoraphobia; the first description of periodic paralysis; the report of a relationship between tabes dorsalis and general paralysis of the insane, prefiguring the syphilis connection; work on pseudosclerosis; and (in 1875) the first description of the deep tendon reflex. In 1887, two years after Ludwig Edinger's description in embryos, he described the accessory nucleus of the 3rd nerve which bears his name. His picture is that of a well-groomed, bearded aristocratic man with a bow tie (Figure 1).

Figure 1 Portrait of Karl Friedrich Otto Westphal (1833–1890). *Source*: From Ref. 22.

Dr. Westphal died in 1890 and is not frequently credited for his report on narcolepsy, which has been linked to the possible forensic implications of sleep attacks (6).

Jean Baptiste Edouard Gélineau had quite a different career, outside of the medical establishment. Born in 1828 close to Bordeaux in the south of France (Blaye, Gascony), he was educated as a navy physician in Rochefort, and practiced on ships, studying tropical disorders in his frequent and long travels to the Indian Ocean. He spent the war as a surgeon-major and was decorated for his services (3). With his large lamb chop beard (Figure 2), it is easy to imagine him with the flamboyant and proud character of people born in the country of Cyrano de Bergerac and of the three Musketeers. Not only was Dr. Gélineau a prolific writer of medical articles and monographs, he also had a great deal of business acumen. Dr. Gélineau was known for his arsenic-bromide tablets to calm neurosis and epilepsy, was involved in coordinating a medical insurance system for older physicians and founded a successful society of health spas and mineral waters. In 1878, he moved to Paris, to rapidly establish a successful private practice, a position he only left in 1900 to retire as a wine grower, owner of the castle of Saint-Luce-La-Tour and seller of Bordeaux wines (probably thanks to the success of his tablets). Dr. Gélineau's publications are eclectic and cover literature, history of his native town, commercial ventures and medical studies. His medical work includes observations on tropical diseases, postpartum psychosis, neurosis, angina pectoris, phobias, deafness, and epilepsy. He is credited for coining the term "narcolepsy" in the attached translated 1880 report, and for forcefully defending it as a unique disease entity distinct from epilepsy. Interestingly, Dr. Gélineau also published a monograph in 1880 on agoraphobia (7), himself citing Wesphal's work on

Figure 2 Portrait and signature of Jean Baptiste Gélineau. *Source*: From Ref. 7.

the topic ("agoraphobie des Allemands"). This indicates knowledge of the work of the German physician prior to his 1880 article or discovered just after his Gazette des Hôpitaux publication. In 1881, Dr. Gélineau also wrote a more detailed account on fourteen narcolepsy cases in a monograph "De la narcolepsie" (8), still not citing Westphal's 1878 narcolepsy report. A careful review of the cases reported in the monograph, however, suggests that most if not all (except the original 1880 case) are not genuine narcolepsy-cataplexy. Whether or not Dr. Gélineau spoke German, and whether the two physicians ever met or corresponded is unknown but not impossible.

IV. Further Comments on Westphal's and Gélineau's Descriptions of Narcolepsy

There is no doubt that both Westphal's and Gélineau's cases have genuine narcolepsy with cataplexy. Both physicians report on the presence of sleepiness and of strange episodes of atonia triggered by emotions, which we now call cataplexy. In both cases, onset was somewhat late in life, 34–36 years old and abrupt, following what could be considered a psychological insult. Earlier reports of narcolepsy have been attributed to Willis (1672, in "De anima brutorum"), Schindler (1829), Bright (1836), Graves (1851), Caffé (1862), and Fischer (1878) but described in fact cases of either isolated severe, overwhelming (narcolepsy-like) sleepiness or atypical/imprecisely described (cataplexy-like) "fits" (9–11). A missing aspect in these reports is the lack of description of automatic behavior, abnormal dreaming and sleep paralysis, which are however neither mandatory nor specific for narcolepsy. Hypnagnogic hallucinations in particular had been described earlier by Alfred Maury (12), and sleep paralysis by Binns in 1842 and by Mitchell in 1876 (13,14), but were not reported in either case herein. Gelineau's and Westphal's reports are remarkable by their diversity and, in both cases, by the certainty of the two authors reporting on a new disease entity [later authors erroneously equated "narcolepsy" with every condition associated with severe daytime sleepiness (15)]. The descriptions are tainted by their schooling and influenced by their time. Nonetheless, nothing better would be written for many years thereafter and it could be argued that the next major discovery was the documented association of narcolepsy with REM sleep onset by Vogel in 1960 (16).

In Wesphal's report, the description of the case is focused on muscle weakness episodes with persistence of consciousness, and in the discussion (translation abbreviated), the author agonized on whether these episodes do or do not represent genuine epilepsy to summarize wisely that it is impossible to conclude for or against this hypothesis. Westphal pointed out correctly the presence of subtle "positive" motor phenomena during cataplexy consisting of "small sporadic nostril contractions" and "slight twitching movements in the face . . . as were movements of the jaw." The precise observation has been confirmed by electrophysiological recordings (17). Emotional triggers are also noted but are not very well described ("mental stimulation of seeing two boys fighting in the street"; "any type of excitation"). Laughter and joking, for example, are not reported as triggers. It is in this context to note that Oppenheim, in his 1902 article on "Lachschlag" (syncope with laughing), while discussing the differential diagnosis of spells associated with laughing, did not mention narcolepsy (18).

Sleep attacks are noted to occur "especially if not engaged in some physical activity, but is sitting quietly, talking or reading" but also "while standing" and "while walking in the street." Sleep attacks while engaged in physical activity are indeed typical, although not specific, for narcolepsy. A relationship and an association of the muscle weakness episodes with sleepiness is emphasized by Westphal, and considered as an extension of the muscle weakness episodes ("at times, however, these attacks do cause the patient to fall asleep"). The German author did not differentiate completely sleep attacks from cataplectic episodes, an ambiguity which may have reflected the simultaneous co-occurrence of both symptoms in his patient (as can occasionally be observed in narcoleptics). This ambiguity may have also reflected, however, Westphal's uncertainty about the true nature of the sleep attacks. It is of interest to note in fact that in Oppenheim's "Lehrbuch der Nervenkrankheiten," the most important German Textbook of Neurology at the beginning of the 19th Century, such episodes were considered to represent episodes of "psychic immobility" with muscle weakness, rather than "true" sleep attacks (19). Insomnia and the absence of any response to potassium bromate were also noted by Westphal in his report.

Further discussion of Westphal's cases, not translated in this report, also attest to the rise of "pre-psychoanalytic" ideas, already evident in Westphal's prior studies on "sexual inversion" and homosexuality (20). Detailed reference and discussion of the case of Van Zastrow, a famous criminal pedophile evaluated by the author in prison, is made. Contrary to what was generally believed in his time, the author was surprised not to find the criminal epileptic (epilepsy was frequently considered at the time to be a sign of "mental degeneration"), but rather an excessively sleepy person who frequently fell asleep in public (this symptom was severe enough that people were laughing about it). A relationship between his sleepiness and his alleged frequent masturbations, repressed homosexuality and an associated shame is suggested. Whether Mr. Van Zastrow had sleep apnea or Klein Levin syndrome is impossible to reconstitute, but narcolepsy is unlikely.

Gélineau's report is somewhat complementary to Westphal's. Its style is more descriptive, "story telling." A potential head trauma two years prior to onset is reported as a possible contributing factor. Whereas Westphal was interested in both the loss of muscle tone and the sleep attacks (as reflected by the title of his communication), Gélineau was more fascinated by sleep attacks during active tasks such as eating and by the existence of refreshing, short naps. Cataplexy is confused with sleep attacks, but its triggers are very well described, that is, playing cards (and having a good hand), smiling at someone poorly dressed in the street, being surprised by a sudden danger, and anticipating the pleasure of a good play in the theater. Most telling is the story of our patient going to the zoo of the Jardin des Plantes and "falling asleep" in front of the monkey's cage when everyone was laughing around him. The patient had up to 200 episodes per day.

A second article follows the initial report where Gélineau excludes potential differential diagnoses including vertigo, epilepsy, agoraphobia, anxiety, meningitis, and sleeping sickness, and concludes that narcolepsy is a unique disease entity. As mentioned above, Gélineau also wrote a monograph reporting on 13 additional cases, none of whom is likely to have genuine narcolepsy. Gélineau described how decreased brain tissue oxygenation and metabolism in the pons, the "site of emotional regulation and dreams" could occur in selected predisposed patients or was caused, in two patients,

by too much sex ("Venus' pleasures"). Decreased oxygenation would be precipitated by emotions, considered as consuming too much oxygen and energy. Gélineau also reports on numerous therapeutic attempts. Therapies aimed at relieving a potential vasomotor abnormality, including picrotoxin and amyl nitrite to induce vasodilation were tried without success. Further trials with apomorphine had no efficacy. Interestingly, he tried to give strychnine, which is now known to block post-synaptic glycinergic transmission, in particular at the spinal motor neuron, where it could antagonize REM sleep-induced atonia, but obtained only a transitory effect. Dr. Gélineau finally recommended to treat the narcoleptic sleepiness with caffeine [as originally suggested by Willis in 1672 (21)], despite the fact it was of little benefit in his only genuine case. A more potent treatment (ephedrine sulfate) than caffeine was suggested by Janota and Daniels about 50 years later (21).

Gélineau considered, in his monograph, that the sleep of narcoleptic patients was deep and devoid of dreams, which suggests, as stressed below, that the 13 other cases were probably not narcoleptic. Importantly however, he introduced the notion still valid today of a duality in narcolepsy, that of sleepiness associated with falls (also called astasia).

References

1. Gélineau JBE. De la narcolepsie. Gazette des Hôpitaux 1880; 53:626–628; 54:635–637.
2. Westphal C. Eigentümliche mit Einschlafen verbundene Anfälle. Archiv für Psychiatrie und Nervenkrankheiten 1877; 7:631–635.
3. Passouant P. Doctor Gélineau (1828–1906): Narcolepsy centennial. Sleep 1981; 4(3):241–246.
4. Henneberg R. Uber genuine Narkolepsie. Neurol Zbl 1916; 30:282–290.
5. Löwenfeld L. Uber genuine Narkolepsie. Neurol Contralb 1916; 35:282–290.
6. Holdorff B. Carl Westphal (1833–1890). J Neurol 2005; 252:1288–1289.
7. Gélineau JBE. De la kénophobie ou la peur des espaces (agoraphobie des Allemands); 1880.
8. Gélineau JBE. De la narcolepsie: Tessier, imprimerie Surgères (Charente-Inferieure); 1881.
9. Furukawa T. Heinrich Bruno Schindler's description of narcolepsy in 1829. Neurology 1987; 37:146.
10. Fischer F. Epileptoide Schlafzustände. Arch J Psychiatr 1878; 8:200–203.
11. Passouant P. The history of narcolepsy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy Proceedings of the First International Symposium on Narcolepsy. Montpellier, France: Specrum Publications, Inc.; 1976:3–13.
12. Maury A. Des hallucinations hypnagogiques ou erreurs des sens dans l'état intermédiaire entre la veille et le sommeil. Ann Med Psy 1848; 11:26–40.
13. Binns E. The anatomy of sleep; or the art of procuring a sound and refreshing slumber at will. London: J Churchill; 1842.
14. Mitchell SW. On some disorders of sleep. Va Med Monthly 1876; 2:769–781.
15. Blocq P. Semeiology of sleep. Brain 1891; 14:112–126.
16. Vogel G. Studies in psychophysiology of dreams III. The dreams of narcolepsy. Archives of general psychiatry 1960; 3:421–428.
17. Rubboli G, d'Orsi G, Zaniboni A, et al. A video-polygraphic analysis of the cataplectic attack. Clin Neurophysiol 2000; 111(Suppl 2):S120–S128.
18. Oppenheim H. Ueber Lachschlag. Monatschrift für Psychiatrie und Neurologie 1902; 11:242–247.
19. Oppenheim H. Lehrbuch der Nervenkrankheiten. Berlin: S.Karger; 1913.
20. Westphal C. Die konträre Sexualempfindung. Symptom eines neuropathischen (psychopathischen) Zustandes. Arch Psychiat Nervenkr 1869; 2:73–108.
21. Lennox WG. Thomas Willis on Narcolepsy. Archives of Neurology and Psychiatry 1939; 41:348–351.
22. http://www.mrcophth.com/ ophthalmologyhalloffame/westphaljpg

The English Translations:
The Original Reports on Narcolepy and Cataplexy by Gélineau and Westphal

---

Gazette of the Civil and Military Hospitals of the Ottoman Empire
Volume 53, pages 626–628 (I), 1880
Volume 54, pages 635–637 (II), 1880
"ON NARCOLEPSY"
By Dr. Gélineau

I.

I am proposing the name narcolepsy (from the Greek "narcosis," drowsiness, and "lambanein," to seize, to take) for a rare neurosis, or at least one that has been little known until now, characterized by a sudden, brief, urgent need to sleep, which recurs at varyingly-spaced, close intervals. This name calls to mind narcolepsy's twofold analogy with drowsiness and catalepsy.

Initially, I believed that the case I had observed (reported below) was the only known instance; however, in Dr. Delasiauve's *Journal de medicine mentale*, nos. 8 and 9, vol. II, 1862, I have just read that Dr. Caffe published an initial case of this sleep neurosis in his *Journal des connaissances medicales pratiques* (August 20, 1862). I am pleased to report this case here, as undeniable proof of its existence.

CASE I. "For more than a year," states Dr. Caffe, "I observed an employee of the Grand Cercle, 16 Boulevard Montmartre, who, because of an irresistible and incessant propensity to sleep, was forced to resign his position. This forty-seven year old man was tall and strong, married, and had always lived soberly. He had no history of illness, and the first external sign was heaviness and half-closure of the eyelids. This drowsiness, which varied in severity depending on circumstances, had affected him for more than four years, coming on while he was standing, sitting, lying down, or while walking. If he woke up, he would fall back to sleep immediately. Even the most pressing hunger did little to divert these effects; his face was somewhat pale and puffy; attitude detached; stupor, mental sluggishness; persistent stoutness; effect on overall health.

Various treatments were unsuccessful, and a stay at the spa at Brides served only to improve his condition, but not result in complete recovery.

Later, after a terrifying emotional experience and illicit excesses (abuse of coitus, masturbation, and alcoholic beverages), he suffered hallucination and meningitic delirium, for which he was intensively treated by Dr. Semelaigne."

CASE II (my own observation). Mr. G., age thirty-eight, a barrel seller with a nervous, volatile temperament, came to my clinic on February 15, 1879.

He had not experienced convulsions in his youth, nor syphilis at a later age. He has two children, the elder of whom, age thirteen, always accompanies him, and the second of whom is only a few months old. G.'s father was nervous, but was free from illness; his mother died of cancer, and his brother of a stomach ulcer. He

drinks moderately. Five years ago, he suffered acute rheumatism in the joints and Herpes tonsurans at the same time.

Three years ago, during a heated argument, he received a violent blow of the fist from the other party, to which he responded by striking his opponent with a drill, after which he was physically apprehended by a policeman and imprisoned; it was a deeply distressing incident.

Finally, a short time later, a log fell on his head, although it did not cause any great pain; and I find no sensitivity at that spot nor any depression worthy of note.

For a long time, this individual experienced no consequential phenomena. Only in the past two years, when laughing out loud or when anticipating a good business deal in his profession, he would feel weakness in his legs, which would buckle under him. Later, when playing cards, if he was dealt a good hand he would freeze, unable to move his arms. His head would nod forward and he would fall asleep. He would wake up a minute later. Soon, the slightest emotion—the sight of his barrels, for example—would be enough to bring on sleep, and since then, this urgent need to sleep has bothered him constantly. When he eats, his meal is interrupted four or five times by the need to rest. His eyelids droop, his hands drop the fork, knife, or glass. He has trouble finishing a sentence, falls asleep. Rubbing his eyes to ward off this sensation while seated is of no avail. His hands fall inert, he is overcome, bends forward, and falls asleep. If he is standing in the street, when this need comes over him, he wavers, stumbles like a drunk, hears people accuse him of drinking and make fun of him. He cannot answer them. Their taunting overwhelms him all the more, and he collapses, instinctively avoiding passing carriages or horses by a final effort. When several people then form a circle around him which always happens in Paris, he hears them or perceives them offering sympathy, and their amiability paralyzes him, affecting him even more and preventing him from getting up.

If he experiences a deep emotion, whether painful or joyous, the need to sleep is even more urgent and sudden. Thus, for example, if he is closing a good business deal, if he sees a friend, if he speaks with a stranger for the first time, or if he receives a good hand while playing cards, he collapses and falls asleep. If he goes to the Jardin des Plantes, near the Monkey House, the place where curiosity-seekers, children's nannies, soldiers, and hecklers usually congregate, he falls asleep seeing this whole laughing crowd around him. A bolting horse, a carriage about to cross his path, or the sight of a person grotesquely dressed who causes him to smile is all he needs to suffer an attack.

At the theater, he falls asleep at the mere thought of the pleasure he is going to experience. He falls asleep again when sitting in his seat, and his son has to shake him and pinch him to pull him out of it. Once the actors come on stage, however, the need disappears; he follows the play with great interest, not collapsing for a single instant, unless a poignant act arouses too great an emotion in him.

Bad weather, particularly the approach of a storm, increases the frequency of these sleep attacks; he has experienced up to two hundred per day.

The only way to pull him out of these attacks is to shake him strongly, or to pinch him. When he becomes violently angry, he sleeps less, but longer and deeper. When he wakes up, he walks straight and firmly, until a new sleep attack comes over him a quarter of an hour later.

I will always remember the way he entered my clinic. He was guided and supported by his son, who held him by the arm. No sooner had he passed through the door of my office and turned his eyes toward me, than, frozen, his gaze glazed over, his eyelids drooped, he staggered, stumbled, and fell asleep, onto a chair; his son spoke to him and shook him hard, after which he began to speak to me.

During his sleep, his pulse, which ranges from 66 to 68 ordinarily, immediately drops to 38 to 60. His pupils, which are highly contracted when awake, are slightly less so when he is asleep. His pupils contract once again when they are raised and brought near the light. The attacks last one to five minutes.

In addition, nothing in him gives evidence of a state of illness; he is calm, at ease. He eats well and his night-time sleep is excellent, waking only once. He has coffee once a day and is not constipated. His sexual desires have diminished considerably. I should repeat that he has just had a child; he says, however, that the child was conceived in a moment when the illness came over him.

A member of the mutual aid society, his card bears the diagnosis *morbus sacer*. He has been treated at his home and at Salpetriere. When he was going there, he fell asleep several times at the door of the hospital, then at the door of the room, and finally, for the third time, confronting the doctor whom he was there to consult. They recommended potassium bromide, subcutaneous injections, hydrotherapy, electricity, and finally, they cauterized the nape of his neck, but, he says, none of this brought any improvement.

When asked to explain his disease and its onset as best he could, he said that he never feels any pain when he is overcome. He merely feels a deep heaviness, an intracranial emptiness, a sort of whirlwind spinning around inside his head, and a heavy weight on his forehead and in back of his eyes. His thoughts dim and fade; his eyelids close half way. He continues to hear, and he is conscious. Finally, his eyelids close completely, and he sleeps. All of this occurs very quickly, so that the normal physiological sleep phase which occurs in progressive periods of five, ten, and twenty minutes, lasts at most a few seconds for him.

If one has him close his eyes and asks him to speak and walk, as is done in cases of ataxia, his voice fades out, he falls asleep and collapses, but without disordered movements. If he enters a dark place, such as a cellar, he also has increased tendency to fall asleep. When he descends a steep street, he has difficulty remaining standing; also, when he pushes a wheelbarrow, with a small cart hitched to him from behind, he pulls it along easily behind him by means of a harness, and he does not fall asleep, probably because his will is more intense at that particular moment.

During his morbid sleep, he never releases any urine or fecal matter. At my office, he has on occasion spoken for a half hour without falling asleep.

His memory is not affected in the least. He is aware of the status of his business, and he is actively involved in taking care of it, but he is always accompanied, because he cannot go out alone without risk of danger. When he works alone, he has fewer attacks than when he is with someone; this is because he enjoys talking, becomes animated and falls asleep.

The intermittent appearance of this illness, its frequency, its lack of resulting injury would place it in the category of a neurosis. The question arises, however, as to whether it should be included under a type already known, or whether it deserves

a place apart in this group that is so large and already so numerous? That is what we shall examine.

First, is this a form of *epilepsy*? I do not think so . . . He does not experience either tonic convulsions or clonic movements. He feels when he is pinched. He is always conscious of what is happening around him. When one shakes him, one can rouse him from his sleep. He does not stammer when he wakes up, and he recovers his intellectual faculties, his senses, and his motility immediately. Moreover, far from overwhelming him, this rest seems to be necessary for him, and appears to give him strength. Finally, his recall is perfect. In addition, potassium bromide, that touchstone of epileptic seizures and epilepsy, has had no positive effect on him. Besides, what epileptic, after one or two hundred spells of dizziness and falls per day, would keep his intelligence and memory intact after two years?

Dr. Semelaigne, however, sought to link his subject's illness to epilepsy . . . (remainder of paragraph deleted).

We reproduce our colleague's opinions in full, but they are not at all convincing. Here is a man who has had continual falls and dizziness for four years, and has never had a full, typical epileptic seizure. He falls, and his drowsiness ceases after the attack; he falls and the *ictus* never causes him to fall stiff, with resultant injuries of the type so common among epileptics. He falls and immediately recovers his wits, his intelligence. Ah! This is because his fall is similar to that of a drunken person or a sleeping child. It is a collapse caused and *preceded* by drowsiness, whereas in the epileptic seizure, sleep *comes after* the fall. Let us add, finally, that Dr. Semelaigne does not mention the one thing that, for us, constitutes the *criterion* of epilepsy from its mildest to its most severe manifestations: the loss of memory, of recollection of what just happened. A subject who remembers and is conscious of what is happening and what happened after an attack of dizziness, an absence, a fall, is not an epileptic.

II.

Can one confuse the affliction from which G. suffers with kenophobia (from the Greek "kenon," the void; "phobeo," I fear), or the fear of open spaces, to use Mr. Legrand du Saulle's term, or agoraphobia, as the Germans put it? Not anymore. Clearly, when crossing a fairly wide street, a square, he is frightened, upset, hesitant. But it is less the view of the open space which affects and frightens him than the fear of being surprised by a carriage, a wagon, or horses. When emotion stops him in his tracks is the moment that sleep overcomes him, and freezes him in place. Also, a person suffering from kenophobia does not fall asleep . . .

One cannot confuse this affliction with vertigo accompanied by syncope, falling, and the loss of consciousness . . . Finally, what a difference between G., sleeping peacefully, blissfully, his face colored, in comparison to the appearance of a livid, frozen man covered with cold sweat and as pale as death, plunged into syncope!

Dr. Casse had attributed this condition of illness to a *serous and passive congestion of the meninges and of the brain.* I assert that this anatomical injury is difficult to reconcile with an intermittent symptom such as sleep that appears and disappears several times a day . . . whereas the idea of a spasm makes it quite easy to explain.

Can this affliction be linked to various degrees of morbid sleep which have been somewhat forgotten in our day, but which the ancients were careful to distinguish in theirs: cataphora, sopor, stupor, coma, carus, and lethargy? The form, duration, and idiotic insensitivity which characterize these last three types make the comparison impossible from the outset.

Perhaps one could associate it with cataphora, and, if one were to consider only the meaning of the Greek words ("kata," down; "pherein," to carry), one would actually believe in a certain analogy between these two types of sleep. But in cataphora, sleep, which is easily interrupted as in the case of G., starts again as soon as one stops speaking to the patient. The sleep is continuous, of a certain duration, and does not include long intervals in which the subject thinks, acts, and works. Finally, cataphora would not be prolonged for years without ending in death or recovery.

As for *sopor* or drowsiness, an intermediate stage between cataphora and coma, the difference is even more marked. The patient, lying on his back, sleeps even more soundly, and cannot be awakened without great effort, and exhibits clearly defined cerebral symptoms, cephalgia, dizziness, loss of memory, akinesia. However, our patient has no symptoms indicating a cerebral illness . . . and ultimately has more waking hours than sleeping.

Confusion with what the English call "sleeping dropsy," which Dr. Nicolas calls "somnosis" and Dr. Dangaix calls "hypnosis," is impossible . . . Dr. Nicolas recently (reports from the Academy of Sciences, issue of May 10, 1880) outlined the progressive and fatal evolution of sleeping sickness from initial drowsiness to death. Sleeping sickness, he says, begins with drowsiness that is completely indistinguishable from normal drowsiness, and its progression is marked by increments that start with deep sleep, followed by longer and longer periods of sleep, until finally the patient does not wake up again. I might add that, being familiar with the work of my friend, Dr. Nicolas, I invited him to examine this patient with me, and that, as a qualified judge of such matters, he immediately rejected any idea of an analogy between these two afflictions.

I had thought of associating the illness with the particular form of nervous condition that was so well described by Morel under the name *emotive delirium*. I found this idea attractive for a short while. In fact, there is no disputing that G. does have a very obvious degree of emotionality, and that this emotionality provokes the attacks . . . Although it is true that the two illnesses appear in response to the slightest of causes, and even the most bizarre, it all adds up to just one effect for G., namely sleep, whereas the scene is quite complex and varied in emotive delirium, accompanied by agitation, anxiety, palpitations, and clouding of the senses, rapid pulse, exaggeration of ideas, and finally automatism. There is nothing of this sort with G. *He falls asleep without suffering*; a subject suffering from emotive delirium . . . *suffers without falling asleep.*

We also do not believe that it can be considered incipient *ataxia* for short periods, because there are no flashes or jerky movements.

The reduction of strength, motility, and the will in G. also made me think of the *neurasthenic* form of *spinal irritation*. However . . . all the facts are in contrast to cases of neurasthenia.

Therefore, I feel justified in designating *narcolepsy* as a specific neurosis, little known until now, and it is good to draw the attention of observers to it.

Let us remember what happened with agoraphobia, which was long confused with vertigo. Once identified, many practitioners in every country throughout the world began to recognize it immediately. Perhaps the same will be true for narcolepsy, which we consider a specific neurosis, characterized by the twofold criterion of drowsiness and falling or astasia . . .

A few words of explanation regarding the cause . . . will help us, I believe, explain the pathogenesis of this neurosis.

Probably, through a special idiosyncrasy, the amount of oxygen accumulated in the nerve centers is in too short supply there, or the oxygen is exhausted too rapidly under the influence of emotions that are too frequent or too strong. The cerebral wear for G. is perhaps greater than in other people, the arterial capillaries too few or too narrow. Perhaps he experiences too rapid an elimination of the regressive products, particularly phosphates.

Whatever the case may be . . . on each occasion, he is neuroparalyzed or, to put it better, neurolyzed, which results in the frequent need to sleep, sleep being the greatest and most powerful restorer of the weakened organism. This opinion is shared by Dr. Delasiauve who, early in his journal, wrote that, "exposed to rapid losses, the nervous system needs to be reimmersed in immobility and rest."

Given this explanation, borrowed from physiology, if we try to determine the exact anatomical location of this neurosis, I believe that, supported by the authority of Dr. Vulpian, we can place it in the annular protuberance. "The annular protuberance," says Dr. Vulpian (1), "must be considered the center of association for emotional movements: whether the excitement comes from the brain or from outside . . . in great emotional expressions, in dreams and in crying, the protuberance plays the most significant role . . . The result is, on the one hand, a momentary paralysis of the cerebrospinal axis, a suspension of nervosity, resulting in astasia and falling and, on the other hand, momentary anemia which, in turn, causes sleep. These two results that constitute narcolepsy are immediate because, in G., there is some sort of shattering of the annular protuberance and cerebral stun.

To complete this observation, I must say something about the treatment that I employed.

Initially . . . I used picrotoxin . . . and I added various bromides to reduce irritability and the reflex action of the cerebrospinal axis.

I must admit that I did not achieve any positive results by using this medication. On the contrary, my patient lost strength and had an increased tendency to sleep. I abandoned that approach.

Along the same lines, I advised that he inhale amyl nitrite vapors poured onto a handkerchief as soon as the narcoleptic attack began . . . We did not overlook the fact that G.'s pulse fell even further, clearly causing an intracranial void, a whirlwind blowing in his head. The use of this medication thus seemed to be indicated . . . But its use did not prevent the attacks, and we then abandoned it, convinced that cerebral anemia played no role in the neurosis at hand.

Then I used subcutaneous injections of apomorphine, which are extolled in Germany . . . without obtaining any positive results.

Then, I decided to turn the symptoms into a medicine, that is, directly fighting the drowsiness. I placed a seton directly on the nape of the neck, which I maintained, and I prescribed grains of caffeine and caffeine valerianate. He improved slightly, but, being

eager for more pronounced results, I was perhaps mistaken in abandoning this medication to consider another idea.

I used strychnine arsenate in progressive doses, and I did not stop until the patient felt tremors in his limbs. I hoped that using this power agent, I would increase the general tone of the economy, fighting the collapses and constant neurolytic exhaustion. At the same time, I had him take phosphates, very tonic food, and warm showers that were revulsant on the spinal column. I even used hypodermic injections of curare. In sum, I did my best to treat the patient aggressively. Nevertheless, I must admit in all humility that by using these methods I barely managed to obtain a few hours of rest and constant work without sleep in the morning and evening. As we both acknowledged that these successes were not in keeping with our mutual efforts, we lost contact, leaving to time and to nature the care of healing or improving this painful neurosis.

Is the ineffectiveness of these remedies one of the characteristics of this neurosis? . . . .

It is clear from what we have said above that the treatment of narcolepsy is entirely open to study. This is one more point of similarity that it shares with the other neuroses, which are so often the stumbling block of our therapeutic means. Whatever the case may be, I am glad to have been able to present this initial study to my colleagues. I am sure that it will result in further studies, for I have already received from a doctor in Lyon all the elements of a third observation of narcolepsy, which I propose to publish somewhat later.

Archives of Psychiatry and Nervous Disorders
Vol. VII
Berlin, 1877
"TWO MEDICAL CASES"Presented at the Berlin Medical and Psychological Society
By Prof. C. Westphal
I. Larvate epileptic attacks many years before the outbreak of a paralytic mental disorder. (pages 622–631).
II. Peculiar attacks associated with falling asleep. (pages 631–635).

II.

Mr. Ehlert, a bookbinder, was admitted to Charité for the first time on July 18, 1871. He has been admitted a few times since then, and is there now. He is 36, and is reported always to have been healthy. Approximately three months before his first admittance, he became ill, he says, as the result of a fit of anger. He had lost his job because of quarreling. After having a few drinks of schnapps (he is reputedly not a drinker), he went home, where he was scolded by his wife. Soon thereafter, he had a brief "fit" (1—1 1/2 minutes), characterized by a loss of speech, or at least an inability to express words clearly. His whole body was trembling (the patient called it "agitation"), so that he had to sit down (he reported that he had an "involuntary compulsion to sit down"). This "agitation" is said to have continued throughout the entire evening. He slept well that night. He says that he felt completely fine the next day, but a similar

condition (in which he lost his capacity to speak and experienced trembling) occurred thereafter at the least mental stimulation, for example, once when he saw two boys fighting in the street and had, in his mind, taken sides with one of them. Headaches and other complaints never occurred in these instances. Thereafter he was employed in a workshop, which was heated even on hot days. He cites these circumstances as the reason for the increased frequency of the attacks. Approximately 10 weeks before his admittance, the attacks changed so that his teeth chattered, speaking was difficult and, if he had anything in his hands, he would have to lay it aside, because he did not have the strength to continue holding it. During these attacks, he was unable to raise his arms. If the attack came upon him while walking or standing, he had to find some means of support, although a cane was sufficient for the purpose. These attacks varied in duration, depending on whether he had exerted himself beforehand. *He did not lose consciousness during these attacks.* He understood everything when spoken to; he was simply unable to respond coherently or fluently. He always had to close his eyes when doing so.

According to the patient, his mother, who had been struck in the head by a falling brick earlier, also suffers from similar attacks. Specifically, her attacks occur while she is sitting quietly, sewing, eating, or while drinking coffee from her saucer, for example. When asked, he expressly stated that these occurrences in his 61-year-old mother were not caused by any type of senility, and that she had been troubled by such attacks frequently earlier on.

I have had the opportunity to observe the attacks in the patient himself on repeated occasions. He had one of these attacks while I was engaged in conversation with him. While he was still speaking, one could see that a certain change had occurred in his facial coloration, his upper eyelids lowered gradually like those of a person falling asleep (during which the eyes roll upward). Then they opened again once or twice, seemingly with great effort, until they finally shut completely, whereupon the patient stopped speaking after murmuring something incomprehensible. His head sank down to his chest, and his brow seemed forcefully knit. Small sporadic nostril contractions were observable, and the patient's appearance was that of a seated person asleep. After a short time (several minutes), the eyebrows relaxed, the patient raised his right arm a few times as if stretching upward, and rubbed his eyes sleepily, like one awakening from slumber. The scene then repeated itself all over again, during which one could observe that, though apparently asleep, the patient hears if one addresses him, since he nods in response to questions directed to him. Afterwards, he also knows everything that was said during the time.

He experiences many such attacks all day long, especially if he is not engaged in some physical activity, but is sitting quietly, talking or reading. However, even when occupied in a physical task he often undergoes these attacks, e.g. while helping wash the dishes. He then sits down on a bench, continues holding the objects that he had in his hand, nods off, and usually returns to his activity a few minutes later. As he says, he has noticed, as corroborated by others, that the attacks certainly usually start at a specific place in a particular situation. For example, from time to time he has to get papers and other objects from the chief attendant's office. Almost always, while standing, he nods off as described above immediately after picking up these objects; he staggers, with his head on his chest and his trunk bent forward like one intoxicated with sleep, from the office out into the corridor. He then proceeds down the corridor,

and after taking a few steps the attack is over. He never drops the objects given to him, but he holds them differently. He does not carry them with outstretched arms, as before, but his arms hang down loose. He does not lose consciousness at all during these attacks. He says that when he enters the office, his spirit becomes uneasy, that he feels a kind of anxiety, and it seems to him as though something had happened to him there before.

The attacks always come on suddenly. When he was a porter, he had such an attack when a man was giving him an order. The man thought that he was drunk, and told a policeman who happened to be there that he wanted him arrested. Meanwhile the attack passed, and the policeman was quite amazed when the patient reasonably explained to him that it was a medical condition. The patient still had time to run after the man, and to ask him for the order again. He further related that once, when he was leaning far forward over the table to get something from the other side, he experienced an attack in that position, and that he stayed in that position until it had passed.

His information about the sensations that he has during these attacks is as follows. His eyes close involuntarily, and he cannot keep them open. If he manages to open them for a moment, he sees a bright light, but cannot make anything out distinctly. At the same time, he loses all strength in his limbs and the ability to speak. He cannot move, and must sit or lean on something. He says that he does not feel tired like someone on the verge of falling asleep. In his mind, it is as though he were thinking of nothing at all, as if his thoughts were wandering completely. He could not provide a more specific description of his mental condition. He says that he does not experience any dizziness. He reports that he hears and understands what is said to him during the attack, but only pays attention to it if it interests him somewhat.

At times, however, these attacks do cause the patient to fall asleep. The falling asleep appears, as it were, to be an extension or increase of the attack. He says that if he can stretch, the attacks do not go to that extreme. During visits, one often finds the patient already asleep, and one can observe him for fairly long periods at a stretch in that condition. The image is exactly that of a person sleeping peacefully in a seated position. By simply calling his name, he can always be awakened, is aware that he had been sleeping, and notes particularly that upon awakening he is immediately lively and alert, not drowsy. He has also experienced this actual falling asleep while walking in the street. Most often, he steps into the gutter or runs into a lamppost or a person, whereby he is suddenly awakened. He has also stayed asleep in the street and a passer-by, tapping him on the shoulder, wakes him saying, "My good man, you're asleep!" Occasionally another attack occurs after he walks about another hundred paces. This falling asleep in the street, says the patient, usually does not happen if he has a specific destination, but occurs more often when he is strolling around quietly and aimlessly.

Aside from what has been described above, the patient also has attacks that he characterizes as more severe. I was witness to one, which he says falls into this category. The patient was brought into the room by an attendant walking behind him. The patient was completely limp, his eyes were closed, and he was staggering like an intoxicated person, and had difficulty in maintaining his balance. Then all support was removed, and the patient stood free, with only a slight swaying motion, but did not fall. During this time, slight twitching movements in the face were observed, as

were movements of the jaw. The eyes were half shut, and the whites of the eyes, which appeared to be rolled up and to the right, remained visible. Respiration was rapid, with sighing. At times it seemed as though the patient was searching for a chair or a seat to hold himself up, but he only made motions with his head that corresponded to such a search, and did not use his eyes. Finally, he was able to reach the edge of a bed, which he then held onto. Toward the end of the attack, he murmured, "Chair," and then said immediately, "Professor, please excuse me while I take a seat," with his eyes still half shut and continued rapid breathing. Although the attack had given the observers the impression that the patient had been unconscious, when asked, he said that he had been fully conscious during the entire attack, and knew exactly which attendant had brought him into the room.

No specific indication of the onset of the attack in this or any form can be determined through observation. The patient himself states quite clearly that any type of excitation, even of the most minimal kind, is very often the trigger for the attacks. He says that they often occur immediately after such excitation.

The patient's intelligence leaves nothing to be desired, and his demeanor is generally calm and reasonable, and no particularly violent outbreaks have ever occurred, as far as we know, although he is easily roused.

Finally, persistent *night-time sleeplessness* must be noted. He says that he spends only a very small portion of the night sleeping, and that the night-time disturbances of other patients are a kind of entertainment for him, rather than making him uncomfortable.

During his first stay at Charité (July 18, 1871 to December 22, 1871), he was treated consistently with potassium bromate, but to no avail.

As is clear from the medical history, the patient attributes the onset of these attacks to a significant emotion. It is also noteworthy that his mother at times falls asleep while performing ordinary chores. However, the patient notes that there is a difference, in that his mother does not lose control of her limbs during the attacks, as he does, but that when she is drinking coffee, for example, the hand bringing the full saucer to her mouth remains in that position, whereas it would be impossible for him to maintain such a position.

- - - - - - - - - -

One is faced with a predicament in attempting to attribute a name to the illness described above. It would be a simple matter to call these episodes "epileptoid" attacks, as well, and I cannot object to the term, if one wishes to lengthen the list of very varied conditions commonly called by that name. This does not advance our understanding at all, however, and the peculiarity of the attacks, to which I need not add any further detail given the exhaustive description above, persists nonetheless.

In this instance. . .one cannot deny that if additional observations should uncover a fairly common occurrence of such "sleep attacks," then we are in the presence of a pathological manifestation of the nervous system, which . . . deserves no less consideration than epileptic or epileptoid attacks. It is evident that for the time being nothing less than a disease of the central nervous system can be concluded . . .

3
Historical Aspects of the Treatment for Narcolepsy

YASUO HISHIKAWA and TETSUO SHIMIZU
Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

I. Introduction

Treatment that had a significant effect on narcolepsy was not found for many years after this disease was first described in the medical literature by Caffé in 1862. Gélineau, who denominated narcolepsy in 1880 after having tried various drugs available at that time, reported that he could not find any means for healing or improving dramatically the distressing condition of narcolepsy (1).

The basic disorder in narcolepsy is timing of sleep waking. Its main symptoms are excessive daytime sleepiness (EDS), irresistible sleep episodes (ISE), nocturnal sleep disruptions, cataplexy, sleep paralysis, and hypnagogic hallucinations. These symptoms are frequently found in many, but not in every patient of narcolepsy.

In the past 75 years, about 20 drugs were reported to be effective for the treatment of narcolepsy, some of them widely used. However, every pharmacologic treatment available was symptomatic, and had to be maintained for many years, often life long. In addition, some drugs (CNS stimulants) were effective for EDS and ISE, but were not effective for the other symptoms including cataplexy. Some other drugs (antidepressants) were effective for cataplexy and other REM sleep related symptoms, but they were not effective for EDS and ISE. For treating nocturnal sleep disruptions, hypnotic drugs were often used. Therefore, depending upon the variety of symptoms found in narcoleptic patients, a combined use of two or three different drugs was often necessary. Even with the most recently developed drugs, pharmacologic treatment has not been satisfying in many patients. Based on this evidence, the importance of psychosocial counseling and behavioral modification of narcoleptic patients has been repeatedly emphasized in recent years.

II. Pharmacologic Treatment

Pharmacologic treatment has been the fundamental means for managing narcoleptic symptoms. The available therapeutic drugs can be classified into three groups. One group consists of CNS stimulants effective for EDS and ISE. Some drugs belonging to this group were found to be effective earlier then those belonging to the second group, consisting of antidepressant drugs effective for controlling the REM sleep related symptoms of cataplexy, sleep paralysis, and hypnagogic hallucinations. The

last group consists of hypnotic drugs for treating nocturnal sleep disruptions. Historical aspects of development of the drugs belonging to the three groups are described separately below.

A. Treatment for EDS and ISE

Coffee and tea, both of which contain caffeine with CNS stimulating action, have long been used by many people in different countries as favorite drinks for dissipating sleepiness and keeping alert. Many narcoleptic patients must have used these drinks repeatedly in daily life. But the effects of coffee and tea were very mild and insufficient for controlling EDS and ISE in narcoleptic patients. Gélineau in 1880 described the very mild and insufficient effects of caffeine granules for treating EDS in narcoleptic patients (1). About 50 years later, ephedrine was introduced by Doyle and Daniels (1931) for the treatment of narcolepsy, but the effect of ephedrine was also very mild compared with the effect of amphetamine, introduced to the treatment of narcolepsy by Prinzmental and Blooming in 1935 (2). Within about 70 years after their report, several other CNS stimulants were found to be effective for treating the EDS and ISE of narcolepsy. They are methamphetamine, pipradrol, methyphenidate, pemoline, mazindol, selegiline (MAO-B-inhibitor, converted in vivo into amphetamine), and modafinil (3–5). Recently, amphetamine, methamphetamine, methylphenidate, and modafinil have been widely used for the treatment of narcolepsy. In actual treatment, one of these CNS stimulants is administered in two divided doses in the morning and at lunchtime, but it must not be administered after the evening as it may disturb nocturnal sleep. The therapeutic effect of the above described CNS stimulants upon cataplexy was absent or very mild, if ever.

B. Treatment for Cataplexy and Other REM Sleep Related Symptoms

Good therapeutic effects of imipramine, a tricyclic antidepressant, on cataplexy were first reported by Akimoto et al. in 1960 (7). They were all neuropsychiatrists, who were usually engaged in the treatment of narcoleptic patients as well as depressed, psychotic patients. Prior to their study with imipramine in narcoleptic patients, they had assumed that the antidepressant might elevate the activity level of the brain in narcoleptic patients producing favorable effects on narcoleptic symptoms, Particularly on EDS and ISE. Their assumption was favorably based upon analogical inference from the therapeutic effect of the drug improving the mood state and analogical inference from the therapeutic effect of the drug improving the mood state and elevating the activity level of depressed, psychotic patients (imipramine was the only antipressant available at that time). Unexpectedly, Akimoto et al. (1960) found in several narcoleptic patients that imipramine had significantly favorable effects in reducing the episodes of cataplexy, but that the drug had no effect at all for controlling EDS and ISE.

Shortly after the above report on imipramine, Hishikawa et al. (1966) confirmed the favorable effect of imipramine and cataplexy. In addition, they found that both imipramine and desmethylimipramine, an antidepressant, were significantly effective for controlling not only cataplexy but also sleep paralysis and hypnagogic hallucinations, but that the both drugs were not effective for EDS and ISE (3). Hishikawa et al. also found that cataplexy was closely related to the abnormal disposition in narcoleptic

patients showing the REM sleep period frequently at sleep onset (sleep onset REM period), and that narcoleptic patients experienced sleep paralysis and hypnagogic hallucination exclusively in the sleep onset REM period (8,9). In addition, they found that both imipramine and desmethylimpramine had potent suppressing effects upon REM sleep (10). Based on these findings, Hishikawa et al. proposed that the good therapeutic effects of the antidepressants (imipramine, desmethylimipramine) were due to their REM sleep suppressing action (3), or that the drug effects were due to suppressing some of the neural activities for REM sleep (11).

After the above reports on imipramine and desmethylimipramine (both of them tricyclic antipressants), many other antidepressants were developed, and some of them found to be effective not only upon cataplexy but also on other REM sleep related symptoms in narcoleptic patients. Later antidepressants found to be effective for narcoleptic symptoms were tricyclic antidepressant (protriptyline, clomipramine); selective serotonin reuptake inhibitor: SSRI (fluoxetime, fluoxamine, zimelidine); selective noradrenalin reuptake inhibitor: SNRI (viloxazine); and selective serotonin/noradrenalin reuptake inhibitor: SSNRI (venlafaxine) (5,6,12).

All of these drugs were originally development as antidepressants for treating depression, and were later found to be effective for treatment of narcoleptic symptoms. They were commonly effective for cataplexy, but not effective for EDS and ISE. The tricyclic antidepressants commonly have serotonin and noradrenalin reuptake inhibitory action together with anticholinergic action. These tricyclic antidepressants often produce different side effects including atropinic side effects. Compared with these tricyclic antidepressants, later developed SSRI, SNRI and SSNRI had reuptake inhibitory action selective for serotonin and/or noradrenelin, but had no anticholinergic action. From these, SSRI, SNRI, and SSNRI were considered to have no atropinic side effects and much fewer side effects than the tricyclic antidepressants.

Some of the above described antidepressants have been widely used for the treatment of narcolepsy. In actual treatment, one of the antidepressants was usually administered in a single dose at bedtime or in two divided doses in the morning and at lunchtime or at bedtime. For treating narcoleptic patients suffering from cataplexy in addition to EDS and ISE, a combined use of an antidepressant and a CNS stimulant was usually performed (3,5). In recent years, clomipramine or protriptyline were more widely used. For patients with troubling side effects due to tricyclic antidepressants, one of the SSRIs should be used instead of the tricyclic antidepressants. It must be noted that abrupt discontinuation of drugs for treating cataplexy may often lead to a rebound increase of the episode of cataplexy or to a continuous incapacitaing state called "status cataplecticus."

C. Treatment for Disrupted Nocturnal Sleep

Narcoleptic patients often suffer from disrupted nocturnal sleep characterized by frequent, vivid dreams and interrupting awakenings. Muscle twitches and periodic limb movements frequently occur in the nocturnal sleep of narcoleptic patients. In former times, barbiturates were often used for treating nocturual sleep disruption. However, tolerance often developed with prolonged use of barbiturates. Because of this, in recent years benzodiazepines or later-developed hypnotics (zopiclone, zolpidem) were often administered at bedtime. For patients suffering from frequent

muscle twitches and periodic limb movements, clonazepam administered at bedtime was found to be helpful. Brouughton and Mamelak (1979) found in narcoleptic patients that gamma-hydroxybutyrate (GHB), a gamma-aminobutyric acid (GABA) precurser, was effective in consolidating nocturnal sleep and in increasing daytime alertness. GHB was also found to be effective in reducing cataplexy (13). In this study, GHB was orally administered in two or three divided doses at bedtime and once or twice on awakening in the middle of the night. It must be remembered that GHB administered in amounts sufficient to induce sleep often gave rise to unusual activity of high amplitude in the humam EEG (14).

III. Psychosocial Counseling and Behavioral Modification

Many narcoleptic patients first receive exact diagnosis and appropriate treatment 10 years or more after the onset of this disease. This was probably because narcolepsy was not well known to patients or to medical doctors in general. Many narcoleptic patients unable to cope with difficulties due to symptoms of the disease often had serious and deleterious effects on work, education, driving, recreation, and family-life. In addition, the effect of pharmacologic treatment was often insufficient for controlling narcoleptic symptoms. especially EDS and ISE. Because of these, many patients were often frustrated and depressed even while on pharmacologic treatment (15). Based on such evidences, many clinical researchers have emphasized the importance of giving narcoleptic patients psychosocial counseling and instructions for behavioral modification prior to and simultaneously with pharmacologic treatment. These were considered to be of great use for improving social adaptation and QOL of narcoleptic patients (5,6,12). Important aspects of psychosocial counseling and behavioral modification advised in the recent years are briefly introduced below.

A. Psychosocial Counseling

Soon after the diagnosis of narcolepsy, all patients and their families should be made aware of that EDS, ISE, and cataplexy are symptoms of a disease called narcolepsy, and that their frequent nappings are not expression of negative attitude or deteriorated behavior due to laziness. In addition, patients should be informed that pharmacologic treatment is available, and that their symptoms will be significantly ameliorated with treatment. These explanations often produce great consolation in many patients, and would significantly alleviate their mental anguish and depression, since they have often been derided and punished for their frequent failure due to EDS and nappings in school and at work (5,6,12,15).

As occupational counseling for narcoleptic patients, it is important to advise them to avoid monotonous and sedentary tasks or jobs that enhance the occurrence of their sleeping episodes. Jobs that require driving for long-distances of shift work, and any job necessitating continuous attention for many hours should be avoided. By marked contrast, occupations that require a continuous level of physical activity can usually be performed adequately by narcoleptic patients (5,6). This information must be given to patients on pharmacologic treatment as well, since EDS and ISE are often refractory to, or insufficiently controlled by, such treatment.

Another important point is to instruct teachers and employers about the nature of the disease to enable them to make appropriate adjustments to schooling and working conditions of narcoleptic patients, and to permit them to take scheduled intermittent rest or brief episodes of sleep (6). A nap for 10 to 15 minuts is often of great use to clear off EDS and to prevent ISE in the following one or two hours.

B. Behavioral Modification

Instruction for behavioral modification should include sleep hygiene and requirements when driving. In general, narcoleptic patients need to keep regular sleep and waking schedules. This is to improve consolidation of nocturnal sleep. Patients with fragmented nocturnal sleep are advised to have sound nocturnal sleep with aid of a hypnotic drug, if necessary. This is important for reducing daytime sleepiness. In addition, it is also important to advise patients to have scheduled short naps three to four times during the daytime. Naps of 10 to 15 minutes are usually very refreshing for most patients. Recommended napping schedules should include naps in mid-morning, soon after lunch, and mid-afternoon. A regular napping schedule will reduce unscheduled EDS and ISE. When narcoleptic patients are adequately treated, driving may be permitted but it must be restricted to short distances. When necessary to drive long-distances, they must stop every one to two hours for a rest or a nap, if necessary (5,6,12).

IV. Conclusions

When reviewing the history of treatment for narcolepsy, we find significant progress but results are not yet satisfying. The goal of treatment for narcolepsy is to maintain patients free of symptoms and side effects of medication. But this goal has rarely been achieved in clinical practice. Physicians caring for narcoleptic patients often must use clinical judgment with a compromise that fits each patient's needs. Patients and clinical doctors both must wait for further progress in the research of therapeutic means for narcolepsy.

References

1. Gélineau J. De la narcolepsie. Gaz d Hop (Paris) 1880; 53:626–628; 54:635–637.
2. Prinzmetal M, Bloomberg W. The use of benzedrine for the treatment of narcolepsy. J Amer Med Ass 1935; 105:2051–2054.
3. Hishikawa Y, Ida H, Nakai K, Kaneko Z. Treatment of narcolepsy with imipramine (Tofranil) and desmethylimipramine (Pertofran). J Neurol Sci 1966; 3:453–461.
4. Hishikawa Y. Stimulant drugs. In: Kales A, ed. Pharmacology of Sleep. Berlin: Springer-Verlag, 1995:421–442.
5. Guilleminault C, Anagnos A. Narcolepsy. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. 3rd ed. Philadelphia: WB Saunders Co, 2000:676–686.
6. Culebras A. Clinical Handbook of Sleep Disorders. Butterworth-Heinemann, Boston, 1996.
7. Akimoto H, Honda Y, Takahashi Y. Pharmacotherapy in narcolepsy. Dis nerv Syst 1960; 21:1–3.
8. Hishikawa Y, Kaneko Z. Electroencephalographic study on narcolepsy. Electroenceph Clin Neurophysiol 1965; 18:249–259.

9. Hishikawa Y, Nan'no H. Tachibana M, Furuya E, Koida H, Kaneko Z. The nature of sleep attack and other symptoms of narcolepsy. Electroenceph Clin Neurophysiol 1968; 24:1–10.
10. Hishikawa Y, Nakai K, Ida H, Kaneko Z. The effect of imipramine, desmethylimipramine and chlorpromazine on sleep wakefulness cycle of the cat. Electroenceph Clin Neurophysiol 1965; 19:518–521.
11. Hishikawa Y, Shimizu T. Physiology of REM sleep, cataplexy and sleep paralysis. In: Fahn S, Hallett M, Lüders HO, Marsden CD, eds. Negative Motor Phenomena. Advances in Neurology. Vol 67, Lippincott-Raven Publishers, Philadelphia, 1995; 245–271.
12. Thorpy MJ, ed. Handbook of Sleep Disorders. Marcel Dekker Inc., New York, 1990.
13. Broughton R, Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. Can J Neurol Sci 1979; 6:1–6.
14. Yamada Y, Yamamoto J, Fujiki A, Hishikawa Y, Kaneko Z. Effect of butyrolactone and gamma-hydroxybutyrate on the EEG and sleep cycle in man. Electroenceph Clin Neurophysiol 1967; 22:558–562.
15. Broughton R, Ghanem Q, Hishikawa Y, Sugita Y, Névsimalova S, Roth B. Life effects of narcolepsy: relationships to geographic origin (North American, Asian and European) and to other patient and illness variables. Canad J Neurol Sci 1983; 10:100–104.

4
Narcolepsy and Hypersomnia: Immunogenetic Aspects of Narcolepsy—Past, Present, and Future

YUTAKA HONDA
Neuropsychiatric Research Institute, Tokyo, Japan

MINAE KAWASHIMA and KATSUSHI TOKUNAGA
Department of Human Genetics, Graduate School of Medicine,
University of Tokyo, Tokyo, Japan

I. Introduction

A genetic component for narcolepsy has been consistently reported across all cultures. Since its initial description by Westphal in 1877, familial narcolepsy has been described by several authors (1,2), suggesting the existence of predisposing genetic factors. Nevsimalova-Bruhova and Roth reported in 1972 that 39.1% of 23 cases of idiopathic hypersomnia and 32.9% of 85 cases of narcolepsy had a positive family history of hypersomnia or narcolepsy (3), and suggested a polyfactorial type of inheritance with two or more genes to explain the transmission of narcolepsy. Kessler in 1976 (4) analyzed 130 narcoleptic families with a narcolepsy proband, and calculated the heritability of narcolepsy to be 0.74. Baraitzer and Parkes (5) analyzed 50 families with a narcolepsy proband and reported that 52% had affected first-degree relatives. Finally, Honda analyzed the mode of inheritance in 232 families with a narcolepsy proband and in 76 families with an excessive daytime sleepiness (EDS) proband (6), and calculated that the heritability of narcolepsy was 0.33 and the heritability of both narcolepsy and EDS was 0.62. There were no narcolepsy patients in families with an EDS proband. Honda proposed a two-threshold multifactorial inheritance model with dominant human leucocyte antigen (HLA)-Dw2 inheritance.

The field of immunogenetic studies in narcolepsy was launched by the discovery of a strong HLA association in narcolepsy in 1984 (7,8). Since then however, there has been no proof for the direct involvement of the immune system in the pathophysiology of narcolepsy. The nucleotide sequences of the HLA-DQ and DR genes were found to be normal in narcoleptic patients (9,10). The frequency of HLA DR2 in narcolepsy was initially reported as 100%, but later there were many reports on the presence of non-DR2 narcoleptic patients, especially among the African-American population. HLA DQB1*0602 was later found to be more significantly associated with narcolepsy across all ethnic groups (11).

In animal models of narcolepsy, it was recently discovered that the nucleotide sequences of the hypocretin/orexin receptor genes were impaired, which resulted in the functional loss of hypocretin/orexin transmission (12,13). But, with the exception

of one patient, no significant changes in the nucleotide sequences of the hypocretin genes were detected in human narcolepsy (14). This suggests that human narcolepsy is not caused by single gene mutations in the hypocretin genes.

The genotype HLA DQB1*0602 remains closely associated with hypocretin-deficient narcolepsy, but its functional role in the brain is still unclear. Direct molecular research of postmortem brains from narcoleptic patients may open new vistas for understanding the mechanisms underlying the cause of narcolepsy. Various research efforts are now being undertaken to further unravel the molecular mechanisms of narcolepsy.

II. The Diagnosis of Narcolepsy and HLA DR2/DRB1*1501 Frequency in Japan

In Japan, serological HLA typing of DR2 in narcolepsy patients started in May 1983. It was followed by serological HLA-DR15 typing in 1987. Four-digit DNA based HLA typing was introduced in April 2001. Non-DR2 narcoleptic patients were rarely found until 2001 when two other physicians joined our sleep clinic . It was recorded that 11.4% of the narcolepsy patients were HLA-DR2-negative. After the founding of the Japan Somnology Center in Tokyo in May 2003, 3 new physicians having less clinical experience with hypersomnia joined our group. As shown in Table 1, the frequency of patients with a diagnosis of narcolepsy but without HLA DRB1*1501/DQB1*0602 increased to 34.5% (16 out of 55 new patients). The diagnosis of narcolepsy was always clinical and at the initial interview. Most diagnoses of HLA-negative narcolepsy patients were made by the newly arrived physicians. This high non-DR2 frequency has recently decreased to 10.1%, suggesting that increased clinical experience in young physicians yielded more homogenous patients.

Table 1 Changes in HLA-DR2 Frequency in Narcolepsy: Effect of Physician Experience in Sleep Medicine

		No. of new narcoleptic pts		No. of physicians and yrs of sleep-clinic experience		
HLA typing method	Period	Total no. of pts	non-DR2/DR15 pts	>20yrs	20>yrs>3	3yrs >
DR2	May 1983–Sept. 1987	206	0 (0 %)	1	0	0
DR15	Oct. 1987–1999	289	2 (0.7 %)	2	0	0
DRB1*1501/DQB1* 0602	2001–June 2003	79	9 (11.4%)	2	1	1
DRB1*1501/DQB1* 0602	July 2003–Dec. 2003	55	19 (34.5%)	2	2	3
DRB1*1501/DQB1* 0602	Jan. 2004–June. 2004	27	7 (25.9%)	2	2	3
DRB1*1501/DQB1*0602	July 2004–Dec. 2004	89	9 (10.1%)	2	1	4

Diagnosis of narcolepsy was made clinically at the initial interview ($n = 745$).

Clinical findings used to diagnose narcolepsy in my clinic include: recurrent daytime sleep episodes of short duration (<30 minutes), associated with feelings of being refreshed and at least five episodes of clinically confirmed cataplexy. Furthersupporting findings include frequent episodes of hypnagogic hallucinations and sleep paralysis; a positive response to psychostimulants and tricyclic antidepressants. Clinical course and onset of EDS and cataplexy are similar in most patients. A narcoleptoid personality" (i.e., decreased psychic tension and alertness) is another important clinical feature (6). A weak familial predisposition is common. These diagnostic criteria were artificially and instinctively created in order to better select a homogenous group of narcoleptic patients. It is most important for immunogenetic studies to use a stricter diagnostic criteria and definition of EDS symptoms.

In our Center, EDS and cataplexy are considered as minimum requirements for the diagnosis of narcolepsy. We also distinguish narcolepsy from essential hypersomnia syndrome (EHS). Our criteria for diagnosing clinical narcolepsy are as follows: (*i*) recurrent daytime sleep episodes, which include naps, lapses into sleep and sleep attacks, occurring basically every day over a period of at least 3 months; (*ii*) clinical confirmation of cataplexy in the patient's history. Cataplexy is defined as a sudden bilateral loss of skeletal muscle tone provoked by a strong emotion. Our diagnostic criteria for EHS are: (*i*) recurrent daytime sleep episodes, which include naps, lapses into sleep and sleep attacks of short duration (<1 hour), occurring basically every day over a period of at least 6 months; (*ii*) absence of cataplexy; (*iii*) diagnostic criteria for other sleep disorders with recurrent excessive daytime sleep episodes, e.g., sleep-apnea syndrome.

Surprisingly, we found that none of the more than one thousand EHS patients we have diagnosed over the past 40 years ever developed cataplexy. It is possible that pharmacological treatment and good sleep hygiene prevented the development of cataplexy. It is also possible that most of the EHS patients without HLA DRB1*1501/DQB1*0602 are etiologically different from narcolepsy. Figure 1 reports on the differential prognosis of EDS in narcolepsy versus EHS patients. These differences may suggest that these two groups of chronic hypersomnia have different causes.

III. HLA Study of Japanese Families with Multiple Narcoleptic Patients

Family and HLA typing studies have also been performed in our population (15). We now have 15 families with more than two patients with definite narcolepsy. The pedigrees with HLA haplotypes of 3 of these families are shown in Figure 2. All narcoleptic and EDS patients and their family members shared the common HLA haplotype, DRB1*1501/DQB1*0602. The severity of the symptoms of narcolepsy tended to decrease in the younger generations when compared to the older generations. In the third generation, patients with mild EDS severity and no cataplexy were observed, supporting a multifactorial model of inheritance for sleepiness. Such forms of EDS in the families may be considered as aborted forms of narcolepsy. Interestingly, one family (Family C) had a chromosomal recombination between the HLA DR-DQ and HLA A-B-C genes (16). The haplotype DRB1*1501/DQB1*0602 was transmitted to the

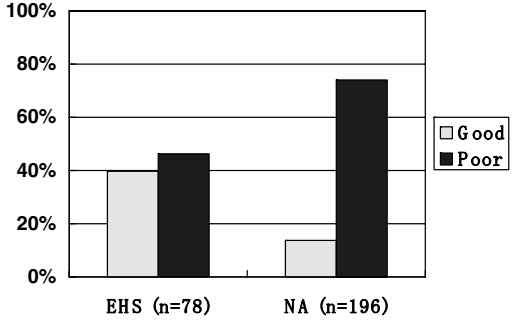
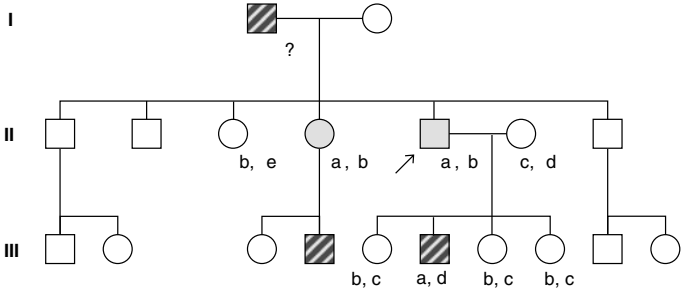


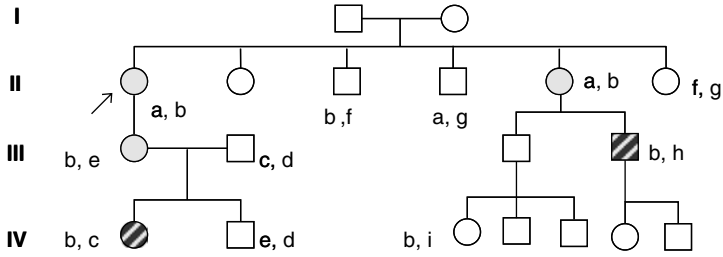
Figure 1 Long-term outcome measures of excessive daytime sleepiness (EDS severity) in narcolepsy and essential hypersomnia syndrome (EHS). Note differences between 196 cases of narcolepsy and 78 cases of EHS for situations without medications over 10 to 40 years. White bars indicate a favorable outcome (marked alleviation of EDS). About 40% of all EHS patients showed absence or rare daytime sleepiness; a similar long-term improvement was observed in only 14% of narcoleptic patients. Black bars indicate poor outcome of EDS after long time course. Note that about 74% of narcolepsy patients still showed frequent EDS in contrast to 46% of EHS. The difference in long-term prognosis indicates etiological difference between narcolepsy and EHS. *Source*: From Ref. 28.



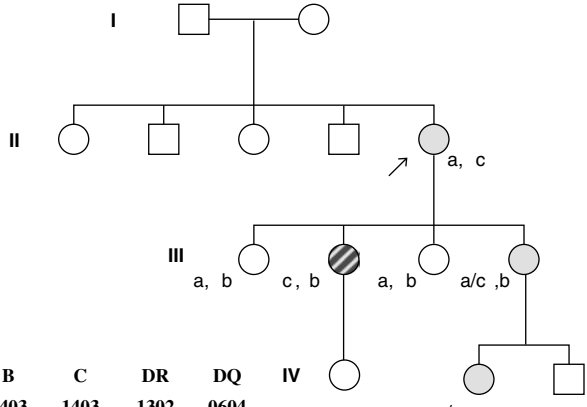
(*a*)

	A	B	C	DR	DQ
a:	0201	5401	0102	1501	0602
b:	0206	5401	0102	0405	0401
c:	1101	1501	0401	0403	0302
d:	0207	4601	0102	0803	0601
e:	3101	4002	0304	1403	0301

Figure 2 Three Japanese multiplex families. Note that all narcoleptic and EDS patients are DRB1*1501/DQB1*0602 positive. Filled boxed indicate narcolepsy and slashed boxes EDS patients. (***a***) Haplotype "a" carries susceptibility to narcolepsy. Note the presence of EDS without cataplexy in the third generation, suggesting decreased severity in descending generations. (***b***) The haplotype "b" carries susceptibility to narcolepsy. A similar decrease of severity was observed in the younger generation. (***c***) The haplotype c carries the susceptibility to narcolepsy. Note the unique translocation of the haplotype DRB1*1501/DQB1*0602 of c with A,B,C of a (a/c) in the second and third generations. The severity of the symptoms of narcolepsy also decrease in the younger generations when compared to the older generations. The patient in the third generation had only mild cataplexy which disappeared later. (*Continued*)



		A	B	C	DR	DQ
	a:	0201	4801	0801	1501	0602
	b:	2603	3501	0303	1501	0602
	c:	2402	0702	0702	0101	0501
	d:	2601	3501	0303	0406	0302
	e:	2402	5401	0102	0405	0401
	f:	0206	5901	0102	0405	0401
	g:	1101	5101	1402	1101	0301
	h:	2602	5401	0102	0405	0401
(*b*)	i:	0207	4601	0102	0901	0303



		A	B	C	DR	DQ
	a:	3303	4403	1403	1302	0604
	b:	2601	4002	0304	0901	0303
	c:	0201	5101	1502	1501	0602
	a/c:	3303	4403	1403	1501	0602
(*c*)	d:	2402	4601	0102	0803	0601

Figure 2 *Continued.*

affected child and grandchild. The recombination breakpoint could be regarded as a boundary for the narcolepsy susceptibility region. Haplotype analyses revealed that the recombination breakpoint was located-50 kb to the telomeric end of the palmitoyl-protein thioesterase-2 gene in the HLA class III region of chromosome 6.

IV. An Independent Association of Tumor Necrosis Factor-Alpha (TNF-Alpha) Promoter Gene Polymorphism in Narcolepsy

Hohjoh performed association studies of tumor necrosis factor-alpha (TNF-alpha) genes in human narcolepsy. The TNF-alpha gene is located in the HLA class III region, a region in linkage disequilibrium with the HLA-DR and DQ genes. Single nucleotide polymorphisms (SNPs) in the promoter region revealed that the frequency of −857T was significantly increased in narcoleptic patients independently of the strong association of DRB1*1501 with narcolepsy. The possibility that TNF-alpha promoter polymorphisms could modulate human narcolepsy susceptibility was suggested (17,18). This was further confirmed in healthy controls who were HLA-DRB1*1501 and DQB1*0602 positive (19). In addition, they found a negative association of DRB1*1502 and a positive association of the TNF-alpha (−857T) and TNF receptor 2 (−196R) combination with narcolepsy. They considered that HLA haplotypes carrying DRB1*1502 could confer a protection against human narcolepsy.

V. Twin Studies of Narcolepsy

Altogether 19 monozygotic narcoleptic twins have been reported in the literature (20,21). Four of 19 pairs (21%) were concordant for narcolepsy (Table 2). Of note, the pair of concordant twins reported by Douglas was HLA-DR2 negative, while another pair recently reported by Khatami had normal cerebrospinal fluid (CSF) hypocretin levels. The other 2 concordant monozygotic pairs had a very different age of onset. Thus, all the reported concordant narcoleptic twins were in some sense atypical.

Table 2 Monozygotic Twin Pairs in the Literature: Human Leucocyte Antigen (HLA) and Hypocretin Status

Author (year)	No. of twins	Concordance	HLA	Hypocretin-1
Imlah (1961)	1	Discordant	ND	ND
Mitchell (1965)	1	Discordant	ND	ND
Mamelak (1979)	1	Discordant	ND	ND
Schrader (1980)	1	Discordant	Dw2	ND
Asaka (1986)	2	Discordant	ND	ND
Montplaisir (1987)	1	Discordant	DR2	ND
Douglas (1989)	1	Concordant	DR4/DQ14	ND
Guilleminault (1989)	1	Discordant	DR2	ND
Pollmacher (1995)	2	Discordant	DR2	ND
Dahlitz (1994)	2	Discordant	DR15	ND
Dahlitz (1996)	1	Concordant	DR15	ND
Hayduk (1996)	1	Discordant	Non-DR2	ND
Honda (2001)	1	Concordant	DQB1*0602	ND
Honda (2003)	1	Discordant	DQB1*0602	ND
Khatami (2004)	1	Concordant	DQB1*0602	546/530
Dauvilliers (2004)	1	Discordant	DQB1*0602	<40/530

The remaining 79% of monozygotic twin pairs with narcolepsy were discordant, and most were HLA DR2 or DQB1*0602 positive. This indicates the important role of environmental factors in the development of narcolepsy, even when there is a HLA-mediated genetic predisposition.

Reporting on our recent experience with two HLA-positive twin pairs (one discordant, one concordant), we found that prolonged sleep deprivation and sustained emotional stress were possible precipitating factors of the onset of narcolepsy (22,23). Our finding is in accordance with previous observations on the role of environmental stress factors prior to the onset of narcolepsy (24). We believe that the study of environmental factors such as stress as possible triggers for narcolepsy may be best investigated in discordant monozygotic twin pairs.

VI. Decreased Hypocretin Levels in the Cerebrospinal Fluid of Narcoleptic Patients

Nishino (25) found undetectable hypocretin-1 (orexin-A) levels in the cerebrospinal fluid (CSF) of narcoleptic patients. Kanbayashi (26) and Krahn (27) found that all narcoleptic patients with both cataplexy and HLA DQB1*0602 showed low CSF hypocretin-1 levels, suggesting that low CSF hypocretin-1 levels and the presence of HLA DQB1*0602 type is closely involved in the development of narcolepsy with cataplexy. On the other hand, there are differences between human and animal narcolepsy models. A rare human patient that had a defect in the nucleotide sequence of the hypocretin gene showed very frequent cataplexy attacks beginning in early childhood (14). The severe cataplexy resembled that observed in canine and murine models of narcolepsy. In human patients, however, the frequency of cataplexy is usually not so much frequent, and narcoleptic symptoms do not appear in early childhood but rather most frequently during adolescence.

In contrast to what is observed in animal models, human narcoleptic symptoms may gradually decline with time. In a follow-up survey using a 185-item questionnaire on the 329 narcoleptic patients who visited our narcolepsy clinic in the past 40 years, we observed that EDS disappeared in 5.2% of the patients, cataplexy stopped in 18.2% of the patients, hypnagogic hallucinations ceased in 35.1% of patients, and sleep paralysis disappeared in 26.0% of patients after 9 years of follow up (Figure 3) (28). This spontaneous improvement was even clearer after a 10 to 39 year follow up where 15.6 % patients had no more EDS, 52.6% patients had no more cataplexy, 56.1% had no more hypnagogic hallucinations, and 62.0% had no more sleep paralysis. The disappearance of cataplexy after a long natural course of illness does not seem to be related to the levels of CSF hypocretin/orexin. Other regulating mechanisms might explain the disappearance of cataplexy and other narcoleptic symptoms, suggesting that hypocretin/orexin deficiency is not the unique determinant of human narcolepsy. Other genetic factors and various cytokines may be involved in the development and spontaneous improvement of narcolepsy.

We previously proposed a two-threshold multifactorial inheritance model with dominant HLA-DR2/DQB1*0602 inheritance as a genetic model for the onset of narcolepsy (6). I wish to add that decreased CSF hypocretin-1 levels could serve as a second threshold for the development of narcolepsy, perhaps as the result

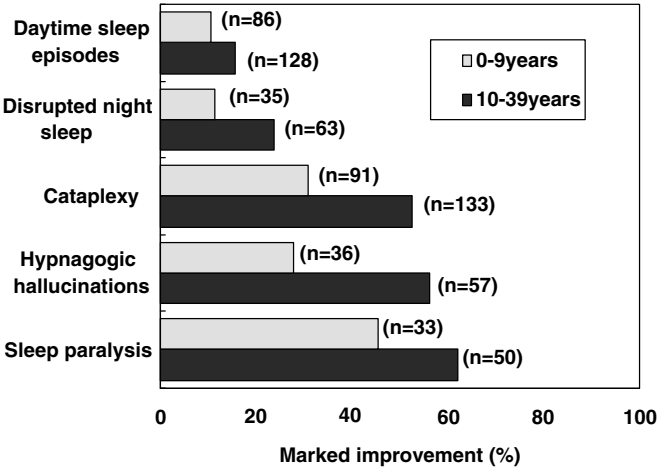


Figure 3 Alleviation of narcoleptic symptoms after 10 to 40 years of follow-up in situations without medication (28). Black bars represent follow-up for less than 10 years, and white bars for 10 to 40 years. A marked alleviation was observed in 15.6% of EDS, and almost half of the cataplexy, hypnagogic hallucinations and sleep paralysis after 10 to 40 years. The so-called REM-related symptoms of narcolepsy showed much a more favorable prognosis than EDS.

of accumulated genetic, psychological, exogenous and other environmental stress factors (Fig. 4).

VII. A Genome-Wide Search for the Susceptibility Genes of Narcolepsy

As mentioned above, genetic factors are important to the development of narcolepsy. Kawashima et al. (29,30) recently conducted a large-scale association study using 25,000 microsatellite markers. A goal was to identify narcolepsy susceptibility regions other than HLA-DR/DQ. The group of narcoleptic patients used for this study was homogenous; they were all Japanese patients living in the Tokyo area, HLA-DRB1*1501/DQB1*0602 positive, and diagnosed by one of us (Y. Honda) by using strict diagnostic criteria. Pooled DNA samples (control and narcolepsy) were first used. One hundred and five narcoleptic patients were pooled for a first set of comparisons, and an additional 110 patients were pooled for a second set of comparison. Similarly, 210 unrelated, healthy controls were pooled for a first set of comparisons, and an additional 210 controls were pooled for a second set of comparisons. Allele frequencies were estimated from peak amplitude differences as detected using an automated sequencer (ABI 3700) and the GeneScan software (Figure 5A). As shown in Figure 5B, markers in the HLA region showed very high peaks of association with narcolepsy, which confirmed the validity of this methodology. Following on the pooled DNA screening, individual typing was performed for the most promising markers. More than 90 microsatellite markers finally showed significant differences using Fisher's exact tests in 2×2 tables.

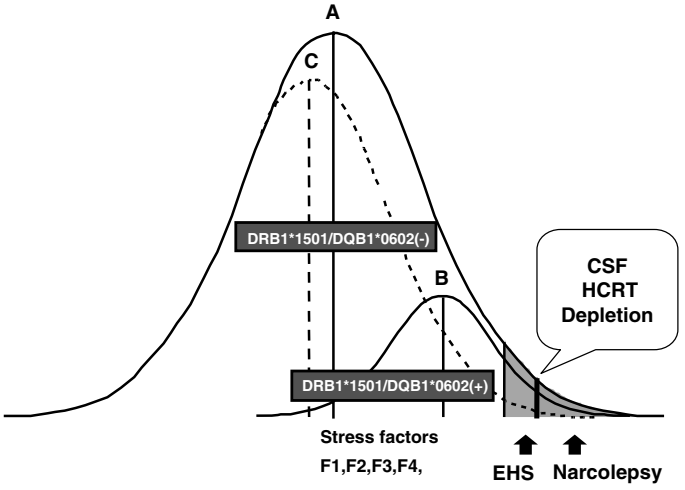


Figure 4 Revised two-threshold multifactorial model with dominant HLA DRB1*1501/DQB1*0602 inheritance and the role of hypocretin for the development of EHS and narcolepsy. The curve A represents the distribution of liability to narcolepsy in the general population. The curve B reports on the distribution of subjects with HLA DQB1*0602. As the sum of genetic (F1), stress (F2), exogenous (F3) and other endogenous (F4) factors accumulate (as shown on the horizontal axis), the liability to narcolepsy increases. They first cross the threshold for EHS and then the second threshold for narcolepsy, usually associated with the depletion of CSF hypocretin.

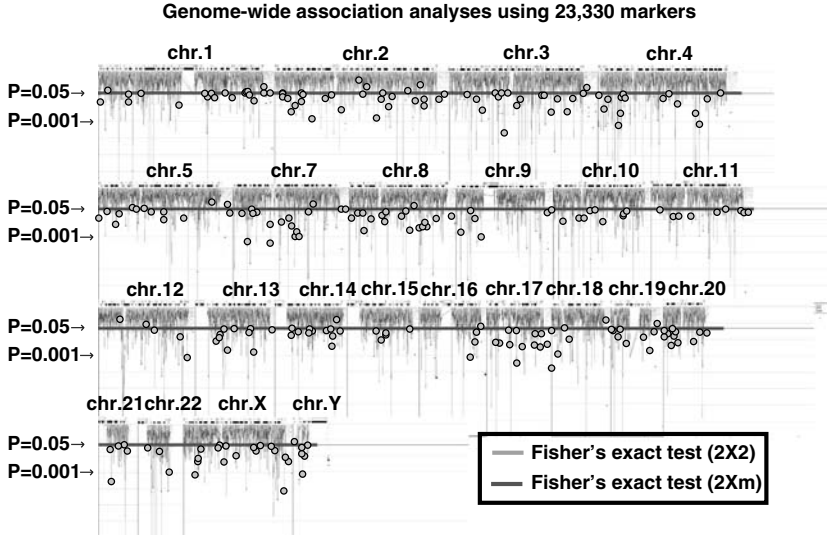


Figure 5 (*a*) Genome-wide analysis of Japanese narcoleptic patients using 25,000 microsatellite markers with pooled DNAs. Significant associations in the first and second screening are shown with large circles. The candidate regions were estimated by the heights of the peaks.

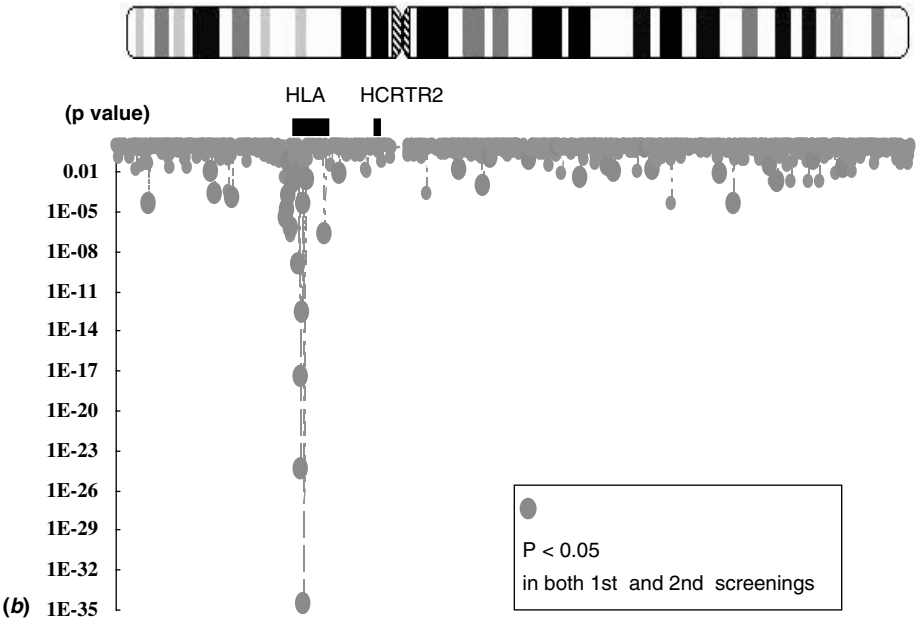


Figure 5 (*b*) Association analysis with 1265 microsatelite markers on chromosome 6. Large circles indicate markers that showed p values of less than 5% in both the first and the second screenings. The markers in the HLA region showed the strongest associations with narcolepsy.

To further narrow down on potential candidates, individual typing was performed on 95 narcoleptics and 95 controls. Three markers, other than HLA, showed strong associations with narcolepsy. These markers also showed strong associations in the analysis with all available samples (228 narcoleptics, 240 controls, $p < 0.001$). These three candidate regions are now subjected to SNP analysis to further narrow down the susceptibility regions of narcolepsy.

Although a slighly different method and a distinct population was used, Wieczorek (31) also used pooled DNA and reported that microsatellites related to various neurotransmitor systems (COMT, DRD2, GABBR1, and HTR2A) were also associated with narcolepsy. Further studies aiming at the identification of susceptibility genes other than those located in the HLA region are needed.

VIII. Postmortem Brain Studies in Narcolepsy

The direct examination of the hypothalamus in the brains of narcoleptic patients is a new approach in narcolepsy research. Peyron (14) used in situ hybridization method and found a loss of hypocretin transcript expression in the hypothalamus. Thannickal (32) reported markedly reduced number of hypocretin immunoreactive cells in the hypothalamus of narcoleptic brains. Makoto Honda and Mignot (33,34) recently performed a molecular search in 8 postmortem brains of narcoleptic patients and compared

them to 6 control brains. They analyzed mRNA expression of 20,000 genes in the hypothalamus. A marked but not complete decrease of the hypocretin signal was observed in the posterior hypothalamus. Interestingly, a preliminary increase in the expression of various immune reactive genes was also found in the anterior hypothalamus. Further analysis is needed to validate and extend on these observations. The interactions among gene products in the hypothalamus may constitute a network of neuroimmunological information processing underlying the mechanisms of sleep. HLA and hypocretin/orexin transmission is closely involved in the pathophysiology of narcolepsy, but how they interact functionally in the brain is still unclear. The direct molecular investigation of postmortem narcoleptic brains may open new avenues, allowing a final understanding of the mechanisms underlying the mysterious cause of narcolepsy.

References

1. Hoff H, Stengel E. Ueber Familiaere Narkolepsie. Klin Wchnschr 1931; 10:1300-1301. (Cited by Luman E, Daniels MD: Narcolepsy. Medicine. 1934; Vol 13:1–122.)
2. Krabbe E, Magnussen G. On narcolepsy: I. Familial narcolepsy. Acta Psychiat Scand 1942; 17:149–173.
3. Nevsimalova-Bruhjova S, Roth B. Heredofamilial aspects of narcolepsy and hypersomnia. Arch Swiss Neurol Neurochir Psychiatr 1972; 110:45–54.
4. Kessler S. Genetic factors in narcolepsy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy. New York: Spectrum, 1976:285–302.
5. Baraitzer M, Parkes JD. Genetic study of narcoleptic syndrome. J Med Genet 1978; 15:254–259.
6. Honda Y. Clinical features of narcolepsy: Japanese experiences. In: Honda Y, Juji T, eds. HLA in Narcolepsy. Heidelberg: Springer, 1988:24–57.
7. Honda Y, Juji T, Satake M. Narcolepsy and HLA: Positive DR2 as a prerequisite for the development of narcolepsy (abstr). Folia Psychiatr Neurol Jpn 1984; 8:3.
8. Juji T, Satake M, Honda Y. Doi Y. HLA antigens in Japanese patients with narcolepsy—All the patients were DR2 positive. Tissue Antigens 1984; 4:316–319.
9. Lock CB, So AKL, Welch KI, Parkes JD, Trowdale J. MHC class II sequences of an HLA-DR2 narcoleptic. Immunogenetics 1988; 27:449–455.
10. Uryu N, Maeda M, Nagata Y, Matsuki K, Juji T, Honda Y, Kasai J, Ando A, Tsuji K, Inoko H. No difference in the nucleotide sequence of the DQ beta 1 domain between narcoleptic and healthy individuals with DR2, Dw2. Hum Immunol 1989; 24:175–181.
11. Mignot E, Lin X, Arrigoni J. DQB1*0602 and DQA1*0102 (DQ1) are better markers than DR2 for narcolepsy in Caucasian and Black Americans. Sleep 1994; 17:S60–S67.
12. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qin X, deJong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–376.
13. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–451.
14. Peyron C, Faraco J, Rogers W, Ripley B, Overeen S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Liu C, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
15. Matsuki K, HondaY, Satake M, Juji T. HLA in narcolepsy in Japan. In: Honda Y, Juji T, eds. HLA in narcolepsy. Heidelberg: Springer, 1988:58–75.
16. Miyagawa T, Hohjoh H, Honda Y, Juji T, Tokunaga K. Identification of a telomeric boundary of the HLA region with potential for predisposition to human narcolepsy. Immunogenetics 2000; 52:12–18.

17. Hojoh H, Terada N, Miki T, Honda Y, Tokunaga K. Haplotype analyses with the human leucocyte antigen and tumor necrosis factor-alpha genes in narcolepsy families. Psychatry Clin Neurosci 2001; 55:37–39.
18. Hohjoh H, Nakayama T, Ohashi J, Miyagawa T, Tanaka H, Akaza T, Honda Y, Juji T, Tokunaga K. Significant association of a single nucleotide polymorphism in the tumor necrosis factor-alpha (TNF-α) gene promoter with human narcolepsy. Tissue Antigens 1999; 54:138–145.
19. Hohjoh H, Terada N, Nakayama T, Kawashima M, Miyagawa T, Honda Y, Tokunaga K. Case-control study with narcoleptic patients and healthy controls who, like the patients, possess both HLA-DRB1*1501 amd DQB1*0602. Tissue Antigens 2001; 57:230–235.
20. Dauvilliers Y, Maret S, Bassetti C, Carlander B, Billiard M, Touchon J, Tafti M. A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology 2004; 11:2137–2138.
21. Khatami R, Maret S, Werth E, Retey J, Schmid D, Maly F, Tafti M, Bassetti C. Monozygotic twins concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 363(9416):1199–1200.
22. Honda M, Honda Y, Uchida S. Monozygotic twins incompletely concordant for narcolepsy. J Biol Psychiatr 2001; 49:943–947.
23. Honda Y. A monozygotic twin pair completely discordant for narcolepsy, with sleep deprivation as a possible precipitating factor. Sleep and Biol Rhthm 2003; 1:147–149.
24. Billiard M, Besset A, Cadilhac J. The clinical and polygraphic development of narcolepsy. In: Guilleminault C, Lugaresi E, eds. Sleep/wake disorders: natural history, epidemiology and long-term evolution. New York: Raven Press, 1983:171–185.
25. Nishino S, Ripley B, Overeem S. Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
26. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, Fujii Y, Nishino S, Shimizu T. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002; 11(1):91–93.
27. Krahn LE, Pankratz VS, Oliver L, Boeve BF, Silber MH. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25(7):733–736.
28. Honda Y. A 10-40 year follow-up study of narcolepsy. In: Meier-Ewert K, Okawa M eds. Sleep-Wake Disorders. NewYork: Plenum Press, 1997:105–114.
29. Kawashima M, Ikuta T, Tamiya G., Hohjoh H, Juji T, Honda Y, Inoko H, Tokunaga K. Genome-wide association study of human narcolepsy: the first and second screening (abst). American Society of Human Genetics, 53rd Annual Meeting, Los Angeles, California, November 4–8, 2003.
30. Kawashima M, Ikuta T, Tamiya G, Hohjoh H, Honda Y, Juji T, Tokunaga K, Inoko H. Genome-wide association study of narcolepsy: Initial screening on chromosome 6. HLA 2004, Immunobiology of the Human MHC. In press.
31. Wieczorek S, Jagiello P, Arning L, Dahmen N, Epplen JT. Screening for candidate gene regions in narcolepsy using a microsatellite based approach and pooled DNA. J Mol Med Aug 7, 2004 (Preliminary on-line report).
32. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
33. Honda M, Salehi A, Hesla P, Maehlen J, Gaus S, Einen M, Mignot E. Gene transcripts relating to the immune response are up-regulated in narcolepsy postmortem hypothalamus using microarray (abst). Sleep 2004; 27:A250–A251.
34. Honda M, Salehi A, Hesla P, Maehlen J, Tanaka S, Einen M, Gaus S, Tsutsumi S, Aburaya H, Honda Y, Mignot E. Microarray analysis of total gene expression pattern in the hypothalamus of narcoleptic patients (abstr in Jpn). 26th Ann Meeting of Jpn Soc of Biol Psychiatr. 260, 2004.

5
The Hypocretins: Discovery and Emerging Role as Integrators of Physiological Signals

LUIS DE LECEA
Departments of Molecular Biology and Neuropharmacology, The Scripps Research Institute, La Jolla, California, U.S.A.[a]

RAPHAËLLE WINSKY-SOMMERER, COVADONGA PAÑEDA, CHISA SUZUKI, and J. GREGOR SUTCLIFFE
Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, U.S.A.

I. Hypocretin Discovery

The hypothalamus plays a central role in the integrated control of feeding, energy homeostasis, circadian rhythms, sex behavior, and arousal. Directional tag PCR subtractive hybridization method was used to identify mRNAs selectively expressed in the hypothalamus. This technology allows comparison of two populations of mRNAs, named target and driver, and isolation of target-specific mRNAs. Briefly, cDNA libraries are prepared from driver and target tissue, and cloned in different plasmid vectors. The target cDNA library is then converted into single stranded cDNA and hybridized with a 50-fold molar excess of in vitro transcribed RNA obtained from the driver library. The hybridization conditions allow rapid annealing of common sequences, and target-specific sequences remain as single stranded cDNAs, which is separated from double stranded cDNA:RNA hybrids by hydroxylapatite chromatography, amplified by PCR and cloned (1). This method has proved successful to identify striatal-enriched cDNAs (1), and to isolate preprocortistatin, a neuropeptide related to somatostatin involved in cortical synchronization and sleep (2).

We determined the sequence of 100 clones of a subtracted library obtained from subtracting hypothalamus versus hippocampus and cerebellum (3). Sequence and expression analysis revealed that they corresponded to 43 distinct mRNA species, about half of which were novel. Thirty-eight of these 43 mRNAs (corresponding to 85 of the clones in the sample) exhibited enrichment in the hypothalamus; 23 were highly enriched. Among the clones showing the highest degree of hypothalamus enrichment were cDNAs for oxytocin, vasopressin, CART, melanin concentrating hormone, POMC, VAT-1, and a novel species called clone 35 (3).

We used the original rat cDNA clone 35 to isolate full-length cDNAs for both rat and mouse. The 569-nucleotide rat sequence suggested that the corresponding mRNA

---

[a]Present address: Department of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, California, U.S.A.

encoded a 130-residue putative secretory protein with four pairs of tandem basic residues for potential proteolytic processing. The mouse homologue sequence differed relative to the rat in only seven coding nucleotides, six of which were near the C-terminus, one of which obliterated a potential proteolytic cleavage site. The absence of this site and the nature of the other differences made it unlikely that two of the four possible rat maturation products were functional. The two remaining putative peptides were absolutely preserved between rat and mouse). Both of these terminated with glycine residues, which in proteolytically processed secretory peptides typically are substrates for peptidylglycine alpha-amidating monooxygenase, leaving a C-terminal amide in the mature peptide. These features suggested that the product of the clone 35 hypothalamic mRNA served as a preprohormone for two C-terminally amidated, secreted peptides. One of these, which was later to be named hypocretin 2 (hcrt2), was, on the basis of the putative preprohormone amino-acid sequence, predicted to contain precisely 28 residues. The other (hcrt1) had a defined predicted amidated C terminus but, because of uncertainties as to how the amino terminus might be proteolytically processed, an undefined N-terminal extent. The C-terminal 19 residues of these two putative peptides shared 13 amino acid identities. This region of one of the peptides contained a 7/7 match with secretin, suggesting that the preprohormone gave rise to two peptide products that were structurally related closely to each other and distantly to secretin. Sequence similarities with various members of the incretin family, especially secretin, suggested that the gene was formed from the secretin gene by three genetic rearrangements: first, a duplication of the secretin gene; second, deletions of the N-terminal portion of the 5′ duplicate and the C-terminal portion of the 3′ duplicate to yield a secretin with its N- and C- termini leap-frogged (circularly permuted); and third, a further duplication of the permuted gene, followed by modifications, to form a secretin derivative that encoded two related hypocretin peptides (Fig. 1).

Further characterization by in situ hybridization of clone 35 revealed that its expression was restricted to a few thousand neurons in the perifornical area of the lateral hypothalamus (Fig. 1*a*) (4). This selective localization of the hypocretins was confirmed at the peptide level using an antibody directed against the peptide precursor. In addition, the hypocretins were detected in large dense core vesicles within the cell body as well as in dendrites and axon terminals, as shown by electron microscopy (Fig. 1*b*).

In parallel, Sakurai and collaborators used an intracellular calcium influx assay with CHO cells in order to identify endogenous ligands of orphan G-protein coupled receptors (GPCR) (5). Hcrt-r1/OX_1R, binds hcrt-1 with high affinity and hcrt-r2 with 100–1000 fold lower affinity. Searching in the databases allowed the identification of a second receptor, hcrt-r2), that binds both hcrt-1 and 2 with high affinity (5). Both receptors are enriched in the brain but they display different distributions (5).

The distribution of hypocretin-containing fibers throughout the central nervous system and the distribution of the receptors led to the hypothesis that these neuropeptides may play a role in the modulation of integrated behaviors. The studies showing that hypocretin mRNA is absent from narcoleptic brains (6), and that hcrt immunoreactivity is highly decreased in narcoleptic hypothalami (7) provide compelling evidence that the main function of the hypocretinergic system is the regulation of arousal circuits. Recent data on the anatomical and functional afferents to hypocretin neurons is increasing our understanding of how this peptidergic system integrates signals and provides behavioral stability.

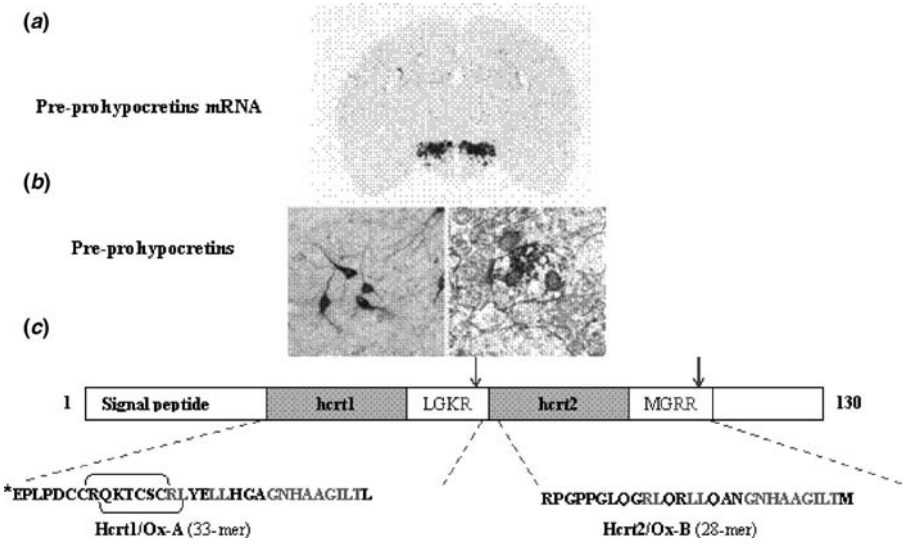


Figure 1 The hypocretin peptides. (***a***) Localization of the pre-prohypocretin mRNA in the rat brain. Labeling is restricted to the perifornical region of the lateral hypothalamic area. (***b***) Hypocretin immunolabeling is observed within neuronal perikarya and in large dense-core vesicles as shown by electron miroscopy. (***c***) Maturation of the preprohypocretin precursor by cleavage at the level of two basic sites to yield Hcrt-1/OX-A and Hcrt-2/OX-B.

II. Hypocretin Neurons Integrate Metabolic Information

The hypocretinergic system has been related to feeding, metabolism, control of body temperature and to the autonomic and endocrine functions. Integration of all this information with the proper state of arousal requires a well-connected system able to sense this variety of signals.

Food intake is regulated by two sets of neurons in the arcuate nucleus, the orexigenic neuropeptide Y (NPY) neurons and the anorexigenic proopiomelanocortin (POMC) containing neurons. In collaboration with Drs Broberger and Hokfelt, we demonstrated that NPY neurons in the arcuate make contacts with hypocretin neurons (8). POMC and MCH neurons also contact hypocretin cells (9). Furthermore, hcrt neurons are sensitive to glucose and activated by low glucose levels (10). Hypocretin neurons do also express leptin receptors, and leptin has been shown to antagonize hcrt effects on NPY and POMC neurons (11). These results suggest that hypocretins serve as integrators of the feeding related signals generated in the adipose tissue, arcuate nucleus and ventromedial hypothalamus.

III. Hypocretins Set the Arousal Threshold

It is well established that absence of hypocretin peptides and neurons leads to arousal instability (12,13). Hypocretin neurons in the lateral hypothalamus receive afferents

from areas critically involved in arousal, including the histaminergic tuberomammilary nucleus (TMN) (14), serotonergic neurons of the median raphe (15) and other brainstem areas. Our current working model postulates that during wakefulness, hypocretin cells receive excitatory input from circadian, metabolic and limbic components, which result in mainly excitatory output to effector arousal nuclei, including the noradrenergic locus coeruleus and histaminergic TMN. During slow wave sleep, activity of hcrt neurons diminishes and results in disinhibition of GABAergic neurons in the ventrolateral preoptic area. During REM sleep, hcrt neurons are silent and disinhibit REM-on neurons in the brainstem (16). In narcolepsy, the absence of hypocretin neurons results in lack of integration and coordination of excitatory signals that modulate wakefulness (17).

Other transmitters such as Neuropeptide S (18), may exert their effects in part through activation of hypocretin neurons. Information from the circadian clock in the suprachiasmatic nucleus may be conveyed indirectly through the dorsomedial hypothalamus.

IV. Hypocretins, Stress, and Addiction

Among the regions innervated by the hypocretin neurons are the ventral tegmental area (VTA), the locus ceorulus (LC), the prefrontal cortex, the hippocampus, the nucleus accumbens, the amygdala, the ventral pallidum and the TMN (14, 19, 20). Defined as the mesocorticolimbic dopamine system, these neurons are related to brain mechanisms of reward, reinforcement, and emotional arousal. In accordance to this, we have investigated whether the hypocretinergic system has a role in the hyperaroused state that is associated with stress and drug addiction. We hypothesized that corticotrophin-releasing factor, a hormone that initiates the stress response and activates the hypothalamo-pituitary-adrenal axis, exerted an effect on hypocretin neurons. Indeed, CRF-containing synaptic terminals contact hypocretin neurons and hypocretin neurons express CRF receptors. Moreover, electrophysiological recordings in hypothalamic slices from transgenic mice that express green fluorescent protein in hypocretin neurons have demonstrated that CRF can depolarize hypocretin cells through CRFR1 receptors. This effect was attenuated, but not blocked in the presence of tetrodotoxin, suggesting both pre- and postsynaptic mechanisms. Further, whereas hypocretin neurons of wild-type mice are activated by acute stress, as measured by c-fosimmunoreactivity, mice deficient in CRFR1 show a dramatic decrease in hypocretin activation after stress. These data suggest that hypocretin neurons are activated by CRF in response to stimuli that cause stress and this activation may be responsible for the extended arousal.

This novel circuitry may have additional implications in drug addiction, since stress is known to promote relapse of drug seeking (21). Using an animal paradigm of cocaine self-administration, we have shown that a single injection of hypocretin can reinstate cocaine seeking behavior in extinguished animals (22).

How could the hypocretinergic system be involved in addiction? As the hypocretin neurons are components of the hypothalamic circuitry that determines most homeostatic set points, alterations in the activity of these neurons can have far-reaching influence on other set points, developing into allostasis. Allostasis is a form of physiological regulation first hypothesized to describe the fluctuations in blood pressure and

immune system function that are not well explained by homeostasis (23). Allostasis represents maintenance of stability at any level outside the normal range and is achieved by varying the internal milieu to match perceived and anticipated environmental demands. Allostasis as a form of regulation allows for the continuous reevaluation and readjustment of all physiological parameters towards new needs as well as for anticipation of such needs and thus, presumably involves the brains control over physiological systems. Thus, in conditions of drug withdrawal, subthreshold stimuli could elicit a pathological response of hyperarousal and drug seeking due to the allostatic threshold set by the hypocretinergic system.

V. Conclusion

Together the existing data in the literature suggest that hypocretin neurons integrate information from multiple, sometimes conflicting systems, and stabilize the networks that promote arousal.

References

1. Usui H, Falk JD, Dopazo A, de Lecea L, Erlander MG, Sutcliffe JG. Isolation of clones of rat striatum-specific mRNAs by directional tag PCR subtraction. J Neurosci 1994; 14(8):4915–4926.
2. de Lecea L, Criado JR, Prospero-Garcia O, Gautvik KM, Schweitzer P, Danielson PE, Dunlop CL, Siggins GR, Henriksen SJ, Sutcliffe JG. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. Nature 1996; 381(6579):242–245.
3. Gautvik KM, de Lecea L, Gautvik VT, Danielson PE, Tranque P, Dopazo A, Bloom FE, Sutcliffe JG. Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. Proc Natl Acad Sci USA 1996; 93(16):8733–8738.
4. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS, 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 1998; 95(1):322–327.
5. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92(4):573–585.
6. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6(9):991–997.
7. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27(3):469–474.
8. Broberger C, de Lecea L, Sutcliffe JG, Hökfelt T. Hypocretin/orexin- and melanin-concentrating hormone expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to neuropeptide Y innervation. J Comp Neurol 1998; 402:460–474.
9. Horvath TL, Diano S, van den Pol AN. Synaptic interaction between hypocretin (Orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: A novel circuit implicated in metabolic and endocrine regulations. J Neurosci 1999; 19(3):1072–1087.
10. Muroya S, Funahashi H, Yamanaka A, Kohno D, Uramura K, Nambu T, Shibahara M, Kuramochi M, Takigawa M, Yanagisawa M, Sakurai T, Shioda S, Yada T. Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca2+ signaling in a reciprocal

manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. Eur J Neurosci 2004; 19(6):1524–1534.
11. Hakansson M, De Lecea L, Sutcliffe JG, Yanagisawa M, Meister B. Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus1 [in process citation]. J Neuroendocrinol 1999; 11(8):653–663.
12. Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 2001; 24:429–458.
13. Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knock-out mice. J Neurosci 2004; 24(28):6291–6300.
14. Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 2001; 21(23):9273–9279.
15. Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, Sakurai T. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. J Neurosci 2004; 24(32):7159–7166.
16. Sutcliffe JG, de Lecea L. The hypocretins: setting the arousal threshold. Nat Rev Neurosci 2002; 3(5):339–349.
17. Mignot E. Sleep, sleep disorders and hypocretin (orexin). Sleep Med 2004; 5 Suppl 1:S2–8.
18. Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, Brucher FA, Zeng J, Ly NK, Henriksen SJ, de Lecea L, Civelli O. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004; 43(4):487–497.
19. Fadel J, Deutch AY. Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. Neuroscience 2002; 111(2):379–387.
20. Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18(23): 9996–10015.
21. Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev 2000; 33(1):13–33.
22. Boutrel B, Kenny PJ, Specio SE, Markou A, Koob GF, de Lecea L. Hypocretin regulates brain reward function and reinstatement of cocaine seeking behavior in rats. Soc Neurosci Abstr 2004:573.572.
23. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann NY Acad Sci 1998; 840:33–44.

6
Cataplexy

CHRISTIAN GUILLEMINAULT, JI HYUN LEE, and VIOLA ARIAS
Stanford University Sleep Disorders Program, Stanford, California, U.S.A.

I. Introduction

The word "cataplexy" was coined by Henneberg in 1916 (1), but many authors described, the sudden loss of muscle tone, under other names. Westphal in 1877 (2) had observed the presence of involuntary movements during motor inhibition that occurred during an abrupt attack; and Gelineau in 1880 (3) had mentioned that emotions may influence sleep attacks and falls or "asbasia". Lowenfeld (1902) is usually credited as the first individual to characterized cataplexy as part of the narcolepsy syndrome (4). Adie (1926) (5) changed Hennebergs term "cataplectic inhibition" to "cataplexy" from the Latin word "cataplessa" which means "to strike down with fear or the like." Daniels in 1934 (6) defined it as "a state of helplessness into which a narcoleptic patient may be precipitated by emotional stress, he is not unconscious but a mass of toneless muscles; and he promptly recovers, non the worse from this experience." Cataplexy, partial or complete, was thus very well described between the end of the 19th and beginning of the 20th centuries. It was linked to narcolepsy. We know now that it may be associated with other clinical entities, but these entities involved the destruction of the hypocretin/orexin neurons in the hypothalamus.

This chapter presents data from patients seen at the Stanford University Sleep Disorders Clinic over 30 years. The cerebrospinal fluid (CSF) hypocretin levels were measured at the Stanford Center for Narcolepsy. The normal values for the laboratory were extracted from normal controls (7). With the technique used in the Center, normal hypocretin levels are considered to be $\geq$200pg/ml, low-abnormal $<$110pg/ml and intermediate level $>$110 $<$200pg/ml.

II. Clinical Characteristics

Cataplexy has been considered pathognomonic of narcolepsy despite the fact that it can be seen exceptionally as an independent problem. Its isolated presence may lead one to question the secondary appearance of daytime sleepiness. Its presence does not allow distinguishing between primary and secondary narcolepsy. As already mentioned by Daniels (6), it consists of a sudden drop of muscle tone triggered by emotional factors, most often by positive emotions, more particularly laughter, and less commonly by negative emotions such as anger. In a review of 200 of our

Table 1 Triggers for Cataplexy in 200 Narcoleptic Cataplectic Patients

Triggers for cataplexy (n = 200) (100%)	
Laughter	100%
Feeling of amusement	82%
Surprise with happiness/joy	78%
Elation	75%
Attempt at repartee	69%
Anger with frustration	57%
Sexual intercourse	38%

Note: These patients were 18 to 30 years of age, *HLA DQB1-0602* positive, and had 2 or more SOREMP at MSLT.

narcoleptics with cataplexy, 100% reported that laughter related to something that subject felt hilarious, triggered an event, surprise with an emotional component was the second most common triggered (see Table 1). Cataplexy occurs more frequently when avoiding taking a nap and feeling sleepy, when emotionally drained or with chronic stress. Elderly subjects with very rare incidence of cataplexy may see a great increase in frequency during a period of grief such as loss of a spouse (8). All striated muscles can be affected leading to a progressive collapse of the subject. Most often the subject with complete collapse has the capability to avoid injury, as the fall is slow and progressive. However one of our reviews of 300 narcoleptics found out occurrence of three skeletal fractures and 27 important bruising related to cataplexy during the first year after diagnosis. Cataplectic attacks may be more limited. It may only involve head and neck, head, neck, and upper limb, more rarely lower limb with "knee buckling" (9). The most common isolated form involves the facial muscles. It leads to a "trembling" of mesenteric muscles, rictus, dysarthria, head and upper arm drop, and drops of object hold in hands (9,10). A study of 40 untreated narcoleptics mean age 16 years (age range 13–23 years) seen for the first time in clinic and asked to keep daily log of cataplectic attacks for a mean of five weeks (range 4–6 weeks) while waiting for polysomnographic recording in our clinic, showed that partial attacks were 13 times more frequent than complete body involvement, in this groups of recently affected pubertal and post-pubertal individuals. Sagging jaw, inclined head, drooping shoulders, and transient buckling of the knees may be the most common presentation. Slurred speech may be noted. Weakness of abdominal muscles and irregular breathing may occur but long diaphragmatic apneas have not been recorded.

The duration of the event is variable but is most often very short. A survey of 100 of our narcoleptics, age between 14 and 24 years, showed that 93% of cataplectic events lasted less than two minutes, with 96% reporting events of 30 seconds or less duration, 6% indicated events lasting up to five minutes, usually when also sleepy. A bit less than 1% (0.93%) reported presence of events longer than five minutes.

The age of onset of cataplexy is variable. In one of our studies of 100 teenagers and young adults (14–23 years) cataplexy was present simultaneously with EDS in 49% of the cases, occurrence of sleepiness between six months and two years after

onset of sleepiness was seen in 41% of subjects. Cataplexy developed between two and six years after sleepiness in 4% of subjects and preceded sleepiness by 0.5 to over three years in 6.0%. Four of the six teenagers had repetitive polysomnographies with 24 hour recording ($n = 1$) or MSLT ($n = 3$). Testings were performed a mean of every four months (ranges 3–5 month) till onset of sleepiness. Cataplexy was seen without evidence of objective sleepiness or presence of sleep onset REM periods (SOREMP) in these cases. Presence of SOREMP was noted only after beginning of complaints of sleepiness that occur between 19 months and 3 years past cataplexy onset. Dauvilliers et al. (11) found that two peaks of symptoms onset were seen in their review, with sleepiness been the leading symptoms. One peak was seen at 15 years and the other at 35 years of age. But these curves of onset of cataplexy did not fit the bimodal distribution of onset of excessive daytime sleepiness (EDS). Despite the presence of an overall bimodal distribution, the age distribution of cataplexy is much wider with onset of cataplexy been reported clearly past 40 years of age. In one of our investigation of 200 narcoleptics, we had report of onset of cataplexy after 45 years of age in 6% of the group.

We have also investigated 51 prepubertal children (12). In 10 of them cataplexy was the initial symptom. The date of onset of EDS may be difficult to pinpoint, however recently a very young child was seen with daytime sleepiness and cataplectic attacks and neurological lesions indicative of a secondary narcolepsy. The mother knew that the child was abnormally sleepy by four months of age, unable to maintain the child awake during feeding or any infant care. Parents may thus recognize very abnormal behavior early. Cataplexy in our young children ($\leq$5 years of age) had been the first symptom and the "drop attacks" seen by parents had been misdiagnosed initially and seizure disorder had been considered and explored initially in all cases. Dauvilliers et al. (13) indicate that early in life appearance of daytime sleepiness and cataplexy is associated with greater severity of the syndrome, after performing a study of two large databases with 519 unrelated and mostly Caucasian narcoleptics.

Cataplexy, overall, has a tendency to decrease with age. An investigation of 100 of our patients that were seen between 12 and 20 years of age, with EDS and cataplexy, showed that after 10 years, 62 had stopped taking anti-cataplectic medications and were kept only on pemoline or methylphenidate for their EDS. A comparison between frequency of cataplectic attacks during first year of diagnosis and 10 years later showered that cataplexy was reported daily in 17%, at least 3 times/week in 57%, and more than 5 times/month in 26% of the cases at entry; but after 10 years cataplexy was reported to occur less than once every 3 months in 41%, and less than 1/month in 28% of the cases. A decrease in frequency of cataplectic attacks with age was also found by Dauvilliers et al. (11) reviewing 383 unrelated mostly Caucasian narcoleptics. This decrease in frequency of cataplexy was less marked, in that study, than the frequency of sleep onset REM periods (SOREMP) at the multiple sleep latency test (MSLT). The explanation for the decrease in frequency of cataplexy is unclear. Some have mentioned a learning behavior with patient avoidance of situation inductive of cataplectic attacks. But this may not cover all situations, as laughter and happy surprise may occur at any age. This decrease, as mentioned above, may be reversed with significant emotional upset, such as grief period in elderly, following family member death.

III. The Secondary Cataplexy

Association of cataplexy with EDS with another disorder of the brain has been reported since the early 1900. The described associations includes tumors, localized most frequently to the diencephalon or to the brain stem, other diencephalic lesions (such as large arterio-venous malformation, or secondary to ischemic events), multiple sclerosis with plaques in the diencephalon, head injury, encephalitis, etc. In young children, Niemann-Pick disease type C, characterized by hepatosplenomegaly, progressive ataxia, dystonia, dementia and vertical supranuclear opthalmoplegia, is often associated with early in life cataplexy, as pointed out by Challamel et al. (14). Cataplexy was noted much earlier in these children with Niemann-Pick, than in our group of prepubertal children (12) with a mean age of onset of 6 years (14–17). The other cause of very early onset of secondary cataplexy is craniopharyngioma. This tumor is one of the most common brain tumors in children and account for 9% of all pediatric intracranial tumors (0.5–2 cases/million population per year) (18). They often present between 5 and 10 years of age. As they grow they can involve the pituitary, optic chiasm, and the hypothalamus. They may lead to severe obesity, hypoventilation, and abrupt bilateral muscle weakness. Resection of the tumor often involves hypothalamic lesions and cataplexy and other symptoms may persist. If the craniopharyngioma has not invaded the hypothalamus, the surgical trauma related to the tumor removal may be responsible for a transient cataplexy that will recede progressively (19) but when cataplexy is present before surgery, removal of the tumor is not associated with regression of cataplexy. With the discovery of the hypocretin/orexin system, and the possibility of measuring CSF Hypocretin-1 (HCRT-1) in patients with cataplexy, EDS and other symptoms associated with narcolepsy, several case reports or short series of neurologic lesions, mostly tumors, have documented that lesions of the lateral and posterior hypothalamus, independently of its mechanism, will lead to lesions of Hcrt producing neurons associated with development of EDS and cataplexy (20–23). Some cases may be a diagnosis challenge. As an example, hypothalamic astrocytoma lead to obesity, pseudo Pradder-Willi syndrome and associated atypical cataplexy. In these secondary cataplexy, the abrupt muscle weakness may not be triggered by laughter (23); and depending on the onset of the neurological syndrome, may be seen very early in life (such as in Niemann-Pick type C (14–17) or late in life. An interesting association has been between development of cataplexy with very little sleepiness associated with clinical symptoms of limbic encephalitis (24). Anti-Ma 2 antibody test was positive. A search for a cancer revealed a testicular cancer. Neurological symptoms precede the diagnosis of cancer in 50% of paraneoplastic syndromes. The presence of cataplexy out of the usual age range, the presence of atypical cataplexy, development of cataplexy without clear association with other symptoms of narcolepsy must raise suspicion and further neurological and other evaluation are warranted not to miss a rare paraneoplastic syndrome and primary cancer site. The existence of an immunologic involvement in the narcolepsy syndrome and paraneoplastic syndromes is an interesting association.

Overall however, the secondary cataplexies are associated with specific lesions located in the lateral and posterior hypothalamus and involving the hypocretin/orexin neurons. These lesions will be seen at brain imaging. Less often neurological lesions will involve the brain stem, interrupting the descending pathways responsible

for maintenance of the active inhibition of the inhibitory reticular formation of Magoun and Rhine. Isolated cataplexy was seen with a pontine pilocystic astrocytoma (25) and with variable EDS with brain stem glioblastoma (26) and subependynoma of fourth ventricle (27). A "status cataplecticus" was reported with a midbrain tumor (28). But the opposite may be true; we had a patient followed for 24 years with a typical narcolepsy-cataplexy that was recognized at 15 years of age and responded to stimulant and tricyclic medications. Cataplexy became very intermittent (less once every three months) with treatment but due to tricyclic side effects, the patient had stopped anti-cataplectic agent. But cataplexy within three months in year 21 of follow-up became again a daily event with multiple triggers not all related to emotions. Frequency and severity of cataplexy had not only changed but response to treatment was limited despite high amount of tricyclic medication. Patient was diagnosed with a slow evolving brain stem glioma. For the following 2 years cataplexy was very poorly controlled. Then with further appearance of brain stem neurological lesions, cataplexy completely disappeared and patient stopped again all medications and had no cataplexy during the last 18 months of his life. Disappearance of cataplexy was simultaneous with medullar region invasion by tumor, as indicated by impairment of 12th cranial nerve. The interpretation of this bimodal evolution was that the brain stem tumor had initially interrupted the descending pathway controlling the active inhibition impinging on the spinal cord motor neurons, and that the neuronal network responsible for this active inhibition was itself progressively lesioned by the tumor. Clinical reports and polysomnographic recordings did not show change in EDS over time with progressive descending extension of the glioma.

The term "status cataplecticus" was first used by Passouant, et al in 1970 (29). It refers to continuous attacks of cataplexy that greatly disable the individual. These cases are rare, we have only observed two over time, they may occurred after abrupt interruption of treatment, but they must not be mixed with the usual rebound of cataplexy that normally occurs between day 5 and 10 after withdrawal of tricyclic or Specific Serotonin Reuptake Inhibitor (SSRI) drugs given to control cataplexy. The attacks are often complete and are triggered by minor situational changes, they usually last longer than the regular attacks and are resistant to the prior administered medications. In our two cases hospitalization was necessary, with isolation from stimulation and it took six weeks to three months to obtain a control of the cataplexy.

IV. The Neurophysiology of Cataplexy

EMG studies and reflexes studies have been performed during cataplexy. Functional MRI studies are attempted during cataplectic attacks, but results are not available. It is considered a negative motor phenomenon (8). One characteristic of cataplexy is the absence of normal jerk reflexes. This transient absence is an important sign of presence of a cataplectic attack. All deep tendon reflexes are back and symmetrical following return of muscle tone. EMG studies performed during complete attacks show that there are short bursts of EMG that will occur on the background of muscle atonia (9,30). These bursts may be seen in one muscle group and not another one (example in upper limb and not in lower limb). They are associated with twitches that are very similar to those monitored during the phasic events of

rapid eye movement (REM) sleep. During the attack patients present dysarthria but can carry on to emit sound, indicating persistence of tone on arrythenoid muscles. Measurement of inspiratory muscles shows presence of short pauses, but these long central apneas are not seen; inhibition last usually no longer than two breathes. Investigation of a monosynaptic reflex—the H-reflex—has been performed in controls and during cataplexy in narcoleptics. There have been several studies that have shown a reduction of H-reflex during laughter in normal subjects (31). Depression of H-reflex has been found with coughing, in ballet dancers when compared to well-trained athletes, and when imaging speed skating in Japanese subjects (32–34). Facilitation has been noted with many influences including jaw mastication or clinching. A recent study compared the effect of laughter and several respiratory movements on H-reflex (31) and it was found that this laughter resulted in a higher H-reflex suppression than simulated laughter, respiratory movement replicating laughter or coughing in normal subjects. The conclusion was that emotional component (mirth) is a much more important than the motor act of laughing to depress H-reflex. In our own study (30), if there were fluctuation at time of H-reflex during a cataplectic attack, when the cataplexy was complete, we had no fluctuation of H-reflex with complete disappearance of the response that lasted up to 3 minutes. There was a long and complete abolition of the H-Reflex. A depression of the H-Reflex was only seen during partial cataplectic attacks, and an incomplete reoccurrence of H-Reflex was only seen when a burst of EMG interested briefly the studied limb (9). Long-lasting complete abolition of the H-reflex without fluctuation was the hallmark of cataplexy and in as such was different from the recordings obtained in normal subjects with laughter.

The narcoleptic dog model has shown that a consistent and large magnitude increase in heart rate prior to cataplexy onset was found (35). It suggested a change in the sympathetic/parasympathetic balance preceding the EMG reduction associated with cataplexy. We performed continuous intra-arterial monitoring of blood pressure with simultaneous ECG recordings and induced cataplexy or monitored spontaneous cataplectic attacks. We observed an increase in blood pressure with onset of cataplexy and a decrease in heart rate, and the heart rate change was secondary to the blood pressure change (36). Change in autonomic control (that may be related to the hypocretin neuron lesion—see below) was however noted in patients with narcolepsy-cataplexy. We reviewed 40 narcoleptics with cataplexy seen between 12 and 24 years of age and shown to be with CSF hypocretin-1 $<$110pg/ml and compared their blood pressure readings with subjects with isolated excessive daytime sleepiness (EDS) We obtained 2 groups with 12 and 15 subjects not statistically different in age, both having sleepiness and$\geq$2 SOREMP at MSLT but one group ($n = 15$) with CSF hypocretin-1 $>$110pg/ml and cataplexy. We found out that subjects with cataplexy had a significant lower systolic and diastolic BP compared to the other subjects [mean systolic $= 93 \pm 0.5$ vs 102 ± 0.9 and diastolic $= 60 \pm 1.8$ vs 64 ± 2 ($p = 0.01$)]. We have also submitted 5 untreated teenagers with recent onset of narcolepsy—cataplexy to tilt test and compared their results to five aged and gender matched controls. We did not induce any cataplexy and had a normal tilt test response, with no significant difference between the two groups.

However, Guilleminault et al. (37) and Aldrich and Rogers (38) have reported several cases of exaggeration of cataplexy with the drug prazosin, a medication

given to lower blood pressure. But the mechanism of action seems unrelated to blood pressure but to the fact that prazosin is an alpha-1 noradrenergic receptor antagonist.

V. Animal Studies

Animal models of cataplexy have proliferated since the discovery of the hypocretin involvement in narcolepsy-cataplexy. But the canine model with its genetic mutation is still the animal model where the largest amount of studies was performed. Wu et al. (39,40) have investigated the activity of monoaminergic cell groups during cataplectic attacks and during sleep in freely moving dogs. Cataplexy was induced by introduction of food, play object or occurred spontaneously. Detailed studies show that noradrenergic cells of the locus coeruleus cease discharge during cataplexy (39), and that serotonergic cells also reduce discharge to NREM sleep level during cataplexy (40). But histamine neurons are active during cataplexy at a level similar to or greater than during quiet waking (41). Also these neurons do not alter their firing with drugs such as Prazosin or physiostigmine that induce cataplexy in dogs. John et al linked the persistence of activity of these histaminergic neurons, located in the tuberomammillary area of the posterior hypothalamus to the maintenance of wakefulness during cataplexy. In human, a low voltage EEG is maintained during cataplexy (9) and subjects are conscious and aware of environment. Dogs present an elevated hippocampal theta during cataplexy (42) but were significantly lower than in REM sleep (41). In summary clear-cut differences in the activity of norepinephrine, serotonin and histaminergic cells were seen during canine cataplexy. Normally all these cells are influenced by the hypocretin neurons, with the locus coeruleus cells receiving predominantly (if not excessively) Hypocretin-1 (Hcrt-1) excitation while histamine cells have predominantly Hcrt-2 influence. The fact that histamine cells carry on firing despite loss of hypocretin neurons is raising some questions and suggests existence of non hypocretin stimulating influence on these histaminergic neurons (41). These findings must be integrated with recent reports by Willis et al. (43) and Kisanuki et al. (44) that showed that cataplexy is more severe in ligand knockout and Hcrt-1 knockout mice than in Hcrt-2 knockout animals that have worse sleepiness. This suggests a different impact of Hchrt-2 and 1 on the symptomatology of narcolepsy; and the persistence of the activity of the histaminergic cells appear to be responsible for that hybrid condition that is, cataplexy with a muscle atonia as in REM sleep and an awake cortex.

VI. Pharmacological Investigations

Human investigations have shown that blockade of reuptake of monoamine particularly noradrenergic reuptake blockade has a beneficial effect on cataplexy. Even medications such as clomipramine or fluoxetine, that have mostly serotonergic reuptake blocking properties, have active metabolites: nor-clomipramine and nor-fluoxetine with noradrenergic reuptake blocking properties. Some stimulants such as dextroamphetamine, metamphetamine have also to a certain degree, such properties; but drugs such as modafinil, that may have more dopaminergic related effect have no influence on

cataplexy that have been shown to rebound when subjects are switch from an amphetamine-like stimulant to modafinil (45). (Note that the mechanism by which gamma-hydroxybutyrate act on cataplexy is unknown to day.)

Animal studies have allowed better understanding of the pharmacology of cataplexy. Muscarinic cholinergic agonists worsen cataplexy while anticholinergic muscarinic substance such as atropine blocks the symptoms (46). Similarly reuptake blockade of noradrenaline has anticataplectic effect (47). Alpha-1 adrenergic antagonists worsen cataplexy while agonist improved it. It is the alpha-1a and 1b receptor subtypes are directly involved in cataplexy (48,49). Dopamine seems to have little involvement despite the fact that it may be involved in the emotional triggering of cataplexy through limbic projection (50). Since the late 70s and early 80s an imbalance between the cholinergic and monoaminergic neurotransmitter systems has been well demonstrated in canine cataplexy, and is well supported also by human data.

VII. Cataplexy and HLA Typing

After the report by J. Juji *et al*. (1984) (51) in Japan of a tight association between narcolepsy and *HLA DR2,* many studies have been performed on patients with EDS and with or without cataplexy. Typing evolved from serological to high-resolution determination. It was found that ethnicity and related presence or absence of linkage disequilibrium between specific alleles had an impact on the major susceptibility allele for the presence of narcolepsy with cataplexy. In Japanese and a high percentage of Caucasians *DQB1-0602* is very tightly associated with *DRB1-1502* (52,53), while in African American and in Martinican with variable mixtures of African and Caucasian origins (54,9,55). The absence of linkage desequilibrium in that group indicated that *DQB1-0602* was the major HLA susceptibility allele for EDS with cataplexy across ethnic groups 55). Depending of the series 88 to 98% of patients with clear cataplexy are *HLA DQB1-0602* independently of ethnicity. Further studies on Caucasians with cataplexy and EDS have shown that, when considering susceptibility for cataplexy and EDS, both *DQA1-0102* and *DQB1-0602* are present suggesting complementation (56) and indicating that these 2 alleles may be important for disease predisposition. *HLA DQB1-0602* homozygotes have two to four times higher risks of developing cataplexy with EDS than heterozygotes (57). Investigations of heterozygoty and different alleles of *DQB1* have shown that some are protective while others favor narcolepsy cataplexy. For example, *DQB1-0601* is protective for the appearance of narcolepsy-cataplexy (58) while higher risk of cataplexy with EDS is seen in heterozygotes expressing also *DQB1-0301*.

As mentioned a large percentage of patients with cataplexy and EDS are *DQB1-0602* and the highest predisposing effect on appearance of cataplexy associate the three locus haplotypes, combination of *DR15-0102*, *DQA1-0102*, and *DQB1-0602*. Eight to 10% of patients with cataplexy and EDS will however be negative for *DQB1-0602* but a high proportion of these patients will carry the susceptibility allele *DQ* B1-0301. In opposition patients with EDS, two or more SOREMP but no cataplexy will have a maximum of 40% chance to carry the major susceptibility allele *DQB1-0602*. This indicates that cataplexy is greatly influence by the presence/absence of specific HLA susceptibility alleles (59).

VIII. Familial Aspect of Cataplexy and HLA

First-degree relations of a narcoleptic patient have 1–2% risk of developing the syndrome, 20 to 40% higher risk than the general population (60). But in our and other studies of multiple families, despite presence of cataplexy, about 30% are *HLA DQB1-0602* negative; these cases indicate that narcolepsy-cataplexy may be related to other genes (60). Vossler et al in 1996 (61) have investigated patients with Norrie disease an X linked dysmorphic syndrome that also associate abrupt falls mimicking cataplexy. They found that in their cases, the Norrie deletion was present, monoamine oxidase (MAO) type B activity was absent and serum serotonin levels were high. This observation indicated a possible link between cataplexy and the monoaminergic pathway. Koch et al in 1999 (62) studied in 28 narcoleptic-cataplectic patients, markers in the Norrie disease region on chromosome X and found a positive association with the intronic variable number of tandem repeat in the *MAO-A* gene. Dauvilliers et al. (2001) (63) performed a case control and a family based association study in 97 Caucasian narcoleptic-cataplectic looking at polymorphisms of *MAO-A* and catechol-O-methyltransferase (COMT). Cataplexy was rated from 1 to 5, a scale related to frequency of cataplectic attacks taken as severity criterion. No evidence of association between genotype or allele frequency and narcolepsy-cataplexy was found. But a sexual dimorphisms and a strong effect of COMT genotype was found on specific symptom severity but not a cataplexy. But it is interesting to note that Niemann-Pick disease type C and Norrie disease with cataplexy clinical presentation are associated with some degree of hypothalamic involvement, as seen in the diencephalic tumors associated with cataplexy.

IX. HLA and Hypocretin/Orexin

With the discovery of the role of the hypocretin/orexin system in animals and humans, and the demonstration of its role in the sleep and wakefulness control, our understanding of narcolepsy-cataplexy has evolved. Measurement of CSF Hypocretin-1 has been available since 2000 (64). Cataplexy when present is key to the diagnostic of narcolepsy when associated with EDS.

In a review of 983 sleep disorders patients and validation of a cataplexy questionnaire (65), we classified patient with "definite" cataplexy, "atypical or doubtful" cataplexy and "no" cataplexy. Based on usage of polysomnography and MSLT, patients with EDS have been also classified with and without two or more SOREMPs. In a review of 410 subjects with EDS, 265 had a history of definite cataplexy. But 44/266(16.5%) had less than two SOREMP AND 73 subjects had no cataplexy and two ore more SOREMP (66) including eight who had five SOREMPs at five nap-MSLT. These results indicate that two or more sleep onset at MSLT may not be necessary for having cataplexy (and narcolepsy). And that the issue of narcolepsy defined only based upon presence of EDS and two or more SOREMP at MSLT is a controversial one; addition of dosage of CSF hypocretin may bring further information (see below). It was found by Hublin, et al. (67), that when cataplexy is present in association with EDS, presence of two or more SOREP during MSLT has a sensitivity of 78.5% and specificity of 62%.

X. The Canine Model of Cataplexy and EDS

The disorder is caused by hypocretin receptor-2 mutation (68). Hypocretin-1 and hypocretin-2 receptor knock-out (KO) mice have cataplexy more marked in the Hcrt receptor-1-KO animals, and anato-pathological studies of brains of patients with narcolepsy-cataplexy were shown to have a global loss of hypocretin neurons, without gliosis or signs of inflammation in one study (68), but presence of residual gliosis in the perifornical region in another (69). These data indicate that cataplexy (and narcolepsy) involves the hypocretin neurons, and that impairment of receptor 2 and/or 1 can lead to cataplexy, depending of the considered animal model.

The availability of dosage of CSF hypocretin-1 in patients with cataplexy and EDS, independently of its etiology that is, sporadic case associated with hypothalamic tumors or other disorders involving the hypothalamus, or presence or absence of ≥ 2 SOREMP at MSLT, has permitted to check for presence/absence of abnormal hypocretin-1 level. It has also allowed to test patients with "atypical or doubtful" cataplexy as identify by the "narcolepsy-cataplexy questionnaire," (65) and also to compare patients with EDS and ≥ 2 SOREMP with and without cataplexy. The results of many CSF measurements performed in the Stanford University Center for Narcolepsy not only demonstrated the validity and accuracy of a direct assay but also determined a threshold of 200 pg/ml. with this technique for normal level in healthy subjects. After performing a quality receiver operating curve (QROC) analysis to determine the most predictive value of CSF-Hcrt-1 for narcolepsy, a cut-off point (low value) of 110 pg/ml was found (7). As reported by Mignot, et al. (2002) (7), out of 106 subjects with narcolepsy 97 (92%) had typical cataplexy, 6 (6%) had "atypical/doubtful cataplexy" and only 3 (3%) had no cataplexy at time of study.

Furthermore, investigation of patients with cataplexy and EDS, related to different hypothalamic lesions but often without SOREMP at MSLT, were shown to have low or absent level of CSF Hypocretin-1 in most cases; (with "low "been defined as: below the cut-off point of 110 pg/ml using the Stanford measurement technique). Sometimes however despite presence of cataplexy, levels are in the intermediate zone i.e., between 110 and 200 pg/ml (Stanford technique). If cataplexy is considered as marker for narcolepsy in patients with EDS, the 110 pg/ml cut-off point has been shown to have a 99% specificity and 87% sensitivity (69). In the same group of subjects used for these calculations, it was noted that 15% of the patients had less than 2 SOREMP at MSLT. Presence of cataplexy was however noted with normal CSF-Hcrt-1 levels in multiplex families. But overall, the presence of "definite cataplexy" means low CSF Hcrt-1 level.

Subjects with cataplexy and *HLA DQB1-0602* have been found to have low CSF Hcrt-1 level in 99% of the cases. On the opposite Mignot, et al (2003) (69) estimated that chance of observing low level of CSF Hcrt-1, in EDS individuals without cataplexy and *HLA DQB1-0602* negative is less than 1%. This would suggest that EDS without cataplexy, normal CSF Hcrt-1, and absence of *HLA DQB1-0602*, is related to a different pathology independently of the number of SOREMP at MSLT.

So presence of cataplexy and *HLA DQB1-0602* is nearly always associated with low level of CSF Hcrt-1. The dosage of CSF hypocretin-1 may thus be useful as a diagnostic test only in presence of atypical doubtful or no cataplexy.

XI. Conclusion

Cataplexy will define most frequently patients that will present both *HLA DQB1-0602* and low to absent CSF Hypocretin-1 level. These patients will often develop their symptoms early during the second decade. They will also have a good chance to have not only cataplexy, but also other symptoms of the narcoleptic tetrad in association with daytime sleepiness and several SOREMP at MSLT testing. They form a homogenous group, and should be recognized as such. It is on this group that effects of homo or hetero-zygosity of associated HLA genes and of the evaluation of variability in severity of syndrome; or studies of response to specific drugs, or to presence of associated symptoms in family members, should initially be performed.

But cataplexy may be seen with EDS particulate in multiplex families, with *HLA DQB1-0602* negative subjects (70), and/or normal or "intermediate" CSF Hypocretin-1 level (7,68), suggesting involvement of other genes than the currently identified HLA gene. The number of SOREMP at MSLT will be variable; and this variable is probably the weakest diagnostic criterion (see below).

Cataplexy may also be seen with EDS in HLA negative patients, but with low CSF Hypocretin-1 levels: These patients have usually an associated lesions of the lateral and/or posterior hypothalamus, or a disorder that is associated with a hypothalamic dysfunction. The only subject identified with a mutation of the preprohypocretin gene is in that subgroup (68). The number of SOREMP will again be variable.

Absence of cataplexy may be seen in narcoleptic patients. This group of subjects is much more heterogeneous than the groups described above: Patients are recognized as "narcoleptics" due to the presence of two or more SOREMP at MSLT. In Mignot, et al. (2002) report (7), 29 of these subjects were identified, 26 had normal CSF Hypocretin-1 levels while only 3 (10%) had low-level CSF Hrct-1.

From other studies, it comes out that a maximum of 40% of subjects with EDS, no cataplexy, and two or more SOREMP at MSLT, will have low level of CSF Hcrt-1, and variable HLA findings. Dauvilliers, et al reported a higher frequency of subjects with low level of CSF Hrct-1 in EDS subjects without cataplexy but positive for HLA DQ B1-0602: out of 22 of such identified subjects 89.5% had low level of CSF Hrct-1 (13).

One may question if subjects with absence of cataplexy and low level of CSF Hrct-1 will not develop cataplexy later in life, but the Dauvilliers et al. group of non-cataplectic subjects had a mean age of 55.25 years (13) giving low plausibility to this hypothesis.

In summary: Cataplexy allows identifying a clear patient group, its absence does not eliminate the diagnosis of Narcolepsy and specific types of impairment; but subjects with absence of cataplexy should trigger a larger investigation and one should not relay only on presence of SOREMP at MSLT, as this recording pattern may be seen with other sleep disorders.

References

1. Henneberg R Uber genuine narcolepsie Neurol Ibe 1916; 30:282–290.
2. Westphal C Eigenthumlich mit einschlafen verbundene anfalle Arch Psychiatr-Nervenks 1877; 7:631–633.

3. Gelineau JB De la narcolepsie Gaz des Hop(Paris) 1880; 53:626–628, 1880; 54:635–637.
4. Lowenfeld L. Uber Narkolepsie. Munch. Med. Wochenschr. 1902; 49:1041–1045.
5. Adie W Idiopathic narcolepsy: a disease sui generic, with remarks on the mechanisms of sleep Brain 1928; 49:257–306.
6. Daniels L Narcolepsy Medicine1934; 13:1–122.
7. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypocretins Arch Neurol 2002; 59:1553–1562.
8. Guilleminault C Gelb M Clinical aspects and features of cataplexy in Negative motor phenomena S Fahn M Hallet H O Ludders CD Marsden (eds) Advances in Neurology vol.67 Philadelphia Lipincott-Raven (publ.) 1995 pp. 65–77.
9. Guilleminault C, Wilson R, Dement WC A study on cataplexy Arch Neurol 1974; 31:255–261.
10. Gelb M, Guilleminault C, Kraemer H, Lin S, Moon S, Dement WC, Mignot E. Stability of cataplexy over several months - information for the design of therapeutic trials Sleep 1994; 17:265–273.
11. Dauvilliers Y, Montplaisir J, Molinari N, Carlander B, Ondze B, Besset A, Billiard M Age at onset of narcolepsy in two large populations of patients in France and in Quebec Neurology 2001; 57:2029–2033.
12. Guilleminault C, Pelayo R Narcolepsy in prepubertal children Ann Neurol 1998; 43:135–142.
13. Dauvilliers Y, Bazin M, Ondze' B, Bera O, Bazin M, Besset A, Billiard M Severity of narcolepsy among French of different ethnic origins Sleep 2002; 25:50–55.
14. Challamel MJ, Mazzola ME, Nevsimalova S, Cannard C, Louis J, Revol M Narcolepsy in children Sleep 1994; 17:S17–S20.
15. Philipart M, Eugel J, Zimmerman E Gelastic cataplexy in Niemann-Pick disease type C and related variants without sphigomyelinase deficiency Ann Neurol 1983; 14:492–493.
16. Kambayashi T, Abe M, Fujimoto S, Miyachi T, Takahashi T, Yano T, Sawaishi Y, Arii J, Szilagyi G, Shimizu T Hypocretin deficiency in Niemann-Pick type C with cataplexy Neuropediatrics 2003; 34: 52–53.
17. Vankova J, Stepanova J, Jech R, Elleder M, Ling L, Mignot E, Nishino S, Nevsimalova S Sleep disturbances and hypocretin deficiency in Niemann-Pick disease type C Sleep 2003; 26:427–430.
18. Einhaus SI, Stanford RA Craniopharyngioma in Albright AL, Pollack IF, Adelson PD (eds) Principles and practice of pediatric neurosurgery New York Thieme 1999; 99:545–562.
19. Schwartz WJ, Stakes JW, Hobson JA Transient cataplexy after removal of a craniopharyngioma Neurology 1984; 34:1372–1375.
20. Malik S, Boeve BF, Krahn LE, Silber MH Narcolepsy associated with other central nervous system disorders Neurology 2001; 57:539–541.
21. Scammell TE, Nishino S, Mignot E, Saper CB Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke Neurology 2001; 56:1751–1753.
22. Arii J, Kambayashi T, Tanabe Y, Ono J, Nishino S, Kohno Y A hypersomnolent girl with decrease CSF hypocretin level after removal of a hypothalamic tumor Neurology 2001; 56:1775–1776.
23. Marcus CL, Trescher WH, Halbowere AC, Luiz J Secondary narcolepsy in children with brain tumor Sleep 2000; 25:435–439.
24. Landolfi JC, Nadkarni M Paraneoplastic limbic encephalitis and possible narcolepsy in a patient with testicular cancer: case study Neuro-oncol 2003; 5:214–215.
25. D'Cruz OF, Vaughn BV, Gold SH, Greenwood RS Symptomatic cataplexy in pontomedullary lesions Neurology 1994; 44:2189–2191.
26. Aldrich MS, Naylor MW. Narcolepsy associated with lesions of the diencephalons. Neurology 1989; 39:1505–1508.
27. Ma TK, Ang LC, Mamelak M, Kish SJ, Young B, Lewis AJ Narcolepsy secondary to 4th ventricle subependynoma Can J Neurol Sci 1996; 23:59–62.
28. Stahl SM, Layzer RB, Aminoff MJ, Towsend JJ, Feldon S Continuous cataplexy in a patient with a mid-brain tumor; the limp man syndrome Neurology 1980; 30:1115–1118.
29. Passouant P, Baldy-Moulinier M, Aussilloux C Etat de mal cataplectique au cours d'une maladie de Gelineau: influence de la clomipramine Rev Neurol (Paris) 1970; 123:56–60.
30. Guilleminault C, Heinzer R, Mignot E, Black J Investigation into the neurological basis of narcolepsy Neurology 1998; 50 suppl1:S8–S15.
31. Overeem S, Taal W, Ocal Geizici E, Lammers GJ, Van Dijk JG Is motor inhibition during laughter due to emotional or respiratory influences? Psychophysiol 2004; 41:254–258.

32. Maillard D, Roby A, Willer JC Depressive effect of coughing on spinal monosynaptic reflexes in conscious man Clin Sci 1983; 65:57–64.
33. Nielsen J Crone C Hultborn H H-reflexes are smaller in dancers from the Royal Danish Ballet than in well-trained athletes Eur J Appl Physiol Occ Med 1993; 66:116–121.
34. Oishi K, Kimura M, Yasukawa M, Yoneda T, Maeshima T. Amplitude reduction of H-reflex during mental movement simulation in elite athletes. Beh. Brain Res. 1994; 62:55–61.
35. Siegel JM, Fahringer HM, Tomaszewski KS, Kaitin K, Kilduff T, Dement WC Heart rate and blood pressure change associated with cataplexy in canine narcolepsy Sleep 1986; 9:216–221.
36. Guilleminault C, Quera-Salva MA, Mancuso J, Hayes B Narcolepsy-cataplexy heart rate and blood pressure Sleep 1986; 9:222–226.
37. Guilleminault C, Mignot E, Aldrich MS Quera-Salva MA, Tiberge M, Partinen M Prazosin is contraindicated in patients with narcolepsy Lancet 1988; 2:511.
38. Aldrich MS, Rogers AE Exacerbation of human cataplexy by prazosin Sleep 1989; 12:254–259.
39. Wu MF, Gulyani S, Yao E, Mignot E, Phan B, Siegel JM Locus coeruleus neurons: cessation of activity during cataplexy Neurosc 1999; 91:1389–1399.
40. Wu MF, John J, Boehmer LN, Yau D, Nguyen GB, Siegel JM Activity of dorsal raphe cells across the sleep-waking cycle and during cataplexy in narcoleptic dogs J Physiol 2004; 554:202–215.
41. John J, Wu MF, Boehmer LN, Siegel JM Cataplexy-active neurons in the hypothalamus; implication for the role of histamine in sleep and waking behavior Neuron 2004; 42:619–634.
42. Kushida CA, Baker TL, Dement WC Electroencephalographic correlates of cataplectic attacks in narcoleptic canines Electroencephal Clin Neurol 1985; 61:61–70.
43. Willie JT, Chemelli RM, Sinton CM Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, ElmquistJK, Kohmeier KA, Yanagigawa M Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes Neuron 2003; 38:715–730.
44. Kisanuki YY, Chemelli RM, Tokita S, Willie JT, Sinton CM, Yanagigawa Behavioral and polysmnographic characterization of orexin-1 and orexin-2 receptor double knock-out mice. Sleep 2001; 24; A22.
45. Guilleminault C, Aftab FA, Karadeniz D, Philip P, Leger D Problems associated with switch to Modafinil-a novel alerting agent in Narcolepsy Eur J Neurology 2000; 7:381–384.
46. Delashaw JB, Foutz AS, Guilleminault C, Dement WC Cholinergic mechanisms and cataplexy in dogs Exp H Neurol 1979; 66:745–757.
47. Foutz AS, Delashaw JB, Guilleminault C, Dement WC Monoaminergic mechanisms and experimental cataplexy Ann Neurol 1981; 10:369–376.
48. Mignot E, Guilleminault C, Bowersox S, Frustofer B, Nishino S, Maddaluno J, Ciaranello R, Dement WC Central alpha-1 adrenoceptor subtypes in narcolepsy-cataplexy a disorder of REM sleep Brain Res 1989; 490:186–191.
49. Mignot E, Renaud A, Nishino S, Arrigoni J, Guilleminault C, Dement WC Canine narcolepsy is preferentially controlled by adrenergic mechanisms: evidence using monoamine selective uptake inhibition and release enhancers Psychopharmacol 1993; 113:76–82.
50. Nishino S, Mignot E Pharmacological aspects of human and canine narcolepsy Pro Neurobiol 1997; 57:27–78.
51. Juji T, Satake M. Honda Y, Doi Y HLA antigens in Japanese patients with narcolepsy Tissue Antigens 1984; 24:316–319.
52. Mignot E, Lin X, Arrigoni J, Macaubas C, Olive F, Hallmayer J, Underhill P, Guilleminault C, Dement WC, Grumet FC DQB1-0602 and DQA1-0102 (Dqw1) are better markers than DR2 for narcolepsy in caucasian and black Americans Sleep 1994; 17:S60–S67.
53. Mignot E, Hayduk R, Black J, Grumet FC, Guilleminault C HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients Sleep 1997; 20(11):1012–20.
54. Neely S, Rosenberg R, Spire J, Antel J, Arnason B HLA antigens in narcolepsy Neurology 1987; 37:1858–1860.
55. Matsuki K, Grumet FC, Lin X, Guilleminault C, Dement WC, Mignot E. HLA DQB1-0602 rather than HLA DRw15 (DR2) is the disease susceptibility gene in Black narcolepsy Lancet 1992; 339:1052.
56. Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Okun M, Hohjoh H, Miki T, Hsu S, Leffell M, Grumet F, Fernandez-Vina M, Honda M, Risch N Complex HLA-DR and DQ interaction confer risk of narcolepsy-cataplexy in three ethnic groups Am J Hum. Genet 2001; 68:686–699.

57. Pelin Z, Guilleminault C, Rich NJ, Grument FC, Mignot E HLA DQB1-0602 homozygousity increases relative risk for narcolepsy but not disease severity in two ethnic groups Tissue Antigens 1998; 51: 96–100.
58. Hungs M, Mignot E Hypocretin/orexin, sleep and narcolepsy Bioessays. 2001; 23:397–408.
59. Chabas D, Taheri S, Renier C, Mignot E The genetics of narcolepsy Annu Rev Genomics Hum Genet 2003; 4:459–483.
60. Guilleminault C, Mignot E, Grumet FC Familial patterns of narcolepsy Lancet 1989; 335:1376–1379.
61. Vossler DG, Wyler AR, Wilkus RJ, Gardner-Walker G, Vlcek BW Cataplexy and monoamine deficiency in Norrie disease Neurology 1996; 46:1258–1261.
62. Koch H, Craig I, Dahlitz M, Denny R, Parkers D Analysis of the monoamine oxydase genes and the Norrie disease gene locus in narcolepsy Lancet 1999; 353:645–646.
63. Dauvilliers Y, Neidhart E, Lecendreny M, Billard M, Tafti M MAO-A and COMT polymorphisms and gene effects in narcolepsy Mol Psychiatr 2001; 6:367–372.
64. Nishino S, Ripley B, Overseem S, Lammers GL, Mignot E Hypocretin (orexin) deficiency in human narcolepsy Lancet 2000; 355:39–40.
65. Anic-Labat S, Guilleminault C, Kraemer HC, Meehan J, Arrigoni J, Mignot E Validation of a cataplexy questionnaire in 983 sleep disorders patients Sleep 1999; 22:7–87.
66. Guilleminault C, Mignot E, Partinen M Controversies in the diagnosis of narcolepsy Sleep 1994; 17:S1–S6.
67. HublinC, Kaprio J, Partinen M, Koskenvuo M, Heikkila K, Kosmikies S, et al. The prevalence of narcolepsy: an epidemiological study of the Finnish twin cohort> Ann. Neurol. 1994; 35:709–716. 1994.
68. Thannickal TC, Moore RY, Nienhauis R, Ramanathan L, Gulyani S, Aldrich M, Conford M, Siegel JM Reduced number of hypocretin neurons in human narcolepsy Neuron 2000; 27:467–474.
69. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Mignot E A mutation in a case of early onset narcolepsy and generalized absence of hypocretin peptides in human narcoleptic brains Nat Med 2000; 6:991–997.
70. Thannickal TC, Siegel JM, Nienhuis R, Moore RY Pattern of hypocretin (orexin) soma and axon loss and gliosis in human narcolepsy Brain Pathol 2003; 13:340–351.
71. Mignot E, Chen W, Black J On the value of measuring CSF-Hypocretin-1 in diagnosing narcolepsy 2003; 26:646–649.
72. Guilleminault C, Grumet FC HLA DR2 and narcolepsy: Not all narcoleptic-cataplectic patients are DR2 Hum Immunol 1986; 17:1–2.
73. Dalal MA, Schuld A, Pollmacher T Undetectable CSF level of orexin-A (hypocretin-1) in a HLA DR2 negative patient with narcolepsy-cataplexy J Sleep Res 2002; 11:273.
74. Khatami R, Maret S, Werth E, Retey J, Schmid D, Maly F, Tafti M, Bassetti C Monozygotic twins concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway Lancet 2004; 363:1199–1200.
75. Neely S Rosenberg R, Spire JP, Antel J, Armason BEW HLA antigens in narcolepsy Neurology 1987; 37:1858–1860.

7
Effect of Age on Narcolepsy

YVES DAUVILLIERS
CHU Montpellier, Unité de Sommeil Hôpital Gui de Chauliac, Montpellier, France

JACQUES MONTPLAISIR
Centre d'Étude du Sommeil, Hôpital du Sacré-Cœur de Montréal, Montréal, Québec, Canada

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

Narcolepsy is a disorder characterized by two major symptoms, namely, excessive daytime sleepiness (EDS) and cataplexy, with a great variability in its presentation. EDS is, in most cases, the first symptom to occur but narcolepsy may start with cataplexy in both adult and prepubertal narcoleptic patients (1). Symptoms may start abruptly with facility to pinpoint the onset of narcolepsy, but it may appear progressively, insidiously, with in some cases sudden worsening of severity. In larger surveys of narcoleptic patients, the mean age at onset was estimated to be in the early 20s. Indeed, Guilleminault et al. (2) studied 410 subjects where the mean age at onset of daytime hypersomnia was 23.7 ± 12.9 years (median age, 20.9 years).

I. Biphasic Distribution of Age at Onset

We did recently a study which focused on the age at onset, in conjunction with severity of narcoleptic symptoms, in two large populations of well-defined cohort of narcoleptics, one from Montpellier-France ($n = 317$) and one from Montreal-Canada ($n = 202$) (3). One interest of looking at narcoleptic populations from Montreal and Montpellier came from that both populations share common genetic background, since over 90% of each population have a French origin. The mean age at onset (23.40 for the French and 24.42 for the Canadian cohort) was quite similar to data already published in the literature. However, the age at onset data were not normally distributed but disclosed two peaks corresponding to an early age at onset of 14.7 years of age and a second peak close to 35 years (Fig. 1). There is no simple explanation for the presence of two peaks of age at onset of narcolepsy. The first peak, occurring between 14 and 15 years of age, may be correlated to the end of puberty. There is evidence that physical or psychological stressors, in addition to genetic predisposition, may contribute to the development of narcolepsy. It is more difficult to pinpoint specific physiological or psychosocial factors that could explain the presence of a second peak in the thirties.

We may also note significant differences for frequency of cataplexy and decreased mean sleep latency on the multiple sleep latency test (MSLT) between

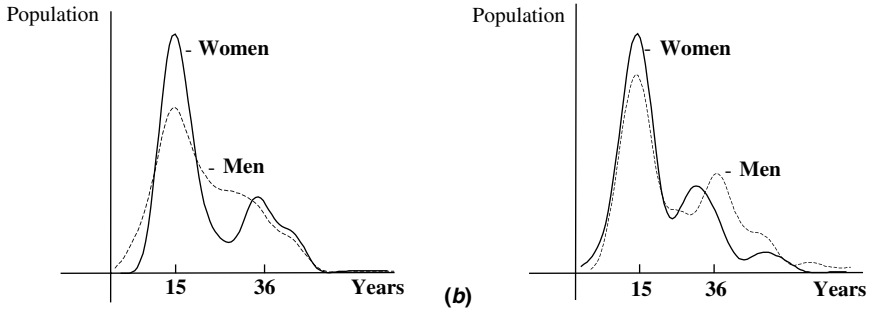


Figure 1 Density curves of distribution of age at onset in the narcoleptic male and female populations, from Montpellier, France (***a***) and Montreal, Canada (***b***).

young and old age at onset in our narcoleptic population, suggesting that early onset narcolepsy may be a factor of severity (3). When looking at the family history, the age at onset clearly separated the patients with a positive family history (early age of onset) from those without a family history. This finding is of importance from a genetic point of view. The earlier the onset of the disease, the stronger is the genetic component. Those findings have also been reported in sleep-onset chronic insomnia and in nonsleep disorder pathologies. Otherwise, most of the other clinical and polygraphic findings were similar in the two populations (Montpellier and Montreal sleep disorder centers). Most of these patients had a similar genetic background but spent their lives in quite different environmental conditions. All these data suggest that the bimodal distribution is an intrinsic characteristic of narcolepsy, possibly genetically determined, rather than a consequence of life events. The presence of two peaks in the age at onset has also been reported in other disorders and especially in autoimmune diseases. Moreover, a young age at onset is frequently associated with high severity of the condition in autoimmune disorders, as reported in the present study (3). These findings reinforce the autoimmune hypothesis (process targeting highly focal hypothalamic hypocretin neurons) in human narcolepsy.

II. Effects of Age on Multiple Sleep Latency Test

Whether the severity of narcolepsy evolves with advancing age is another issue of this review. There is some evidence that age may influence the MSLT results in narcolepsy. Although several methods have been used to assess EDS, MSLT is the most commonly used in the case of narcolepsy. A mean sleep latency lower than five minutes was considered to be a reliable indicator of pathological daytime sleepiness, however values lower than eight minutes are actually being used to define pathological sleepiness (4). A large survey of narcoleptic patients indicated that 87% had a mean sleep latency on the MLST lower than five minutes and 93% lower than eight minutes (5). In addition to short sleep latencies, MSLTs of narcoleptic patients are characterized by the presence of sleep onset REM periods (SOREMPs). Two large studies of narcoleptic patients found that 83% and 74% of patients had at least two SOREMPs during the MSLT, 11%

and 13% showing one SOREMP, and 6% and 13% having no SOREMP (2,5). Several studies have shown that age may influence the MSLT results: indeed children with EDS and cataplexy have markedly reduced sleep latency and a high prevalence of SOREMPs during the MSLT (1,6). Nevertheless, one study looking at the MSLT data of 228 narcoleptics revealed a decrease in total sleep time, sleep efficiency, stages 3 and 4 and REM sleep across the decades, in contrast to an increase in wake time after sleep onset, number of awakenings and percentage of stage 1, but without any difference in EDS and number of SOREMPs (7). However, only adult patients (>20 y.o.) with at least two SOREMPs and a sleep latency shorter or equal to six minutes were included, and the MSLT consisted of only four naps scheduled at a two-hour intervals, (7) all characteristics that may have masked the age differences. Another study failed to observe significant differences in mean age at onset in narcoleptic subjects with <2 SOREMPs compared to those with ≥2 SOREMPs, however, no stratification of age groups was performed and mean sleep latencies were not studied (2).

We did recently a study which focused on the effect of age on MSLT characteristics, that is, sleep latency and number of SOREMPs, in two populations of narcoleptic patients diagnosed on the basis of EDS, cataplexy and the presence of the HLA-DR2 antigen, one in Montpellier-France ($n = 236$) and one in Montreal-Canada ($n = 147$) (8). All patients had been withdrawn from medication known to influence sleep or narcolepsy symptoms. The results showed a significant progressive decrease in the number of SOREMPs with age and a progressive increase in the mean sleep latency on the MSLT as a function of age. This finding is also related to the frequency of cataplexy as assessed from the clinical history with a progressively decrease with age. The results of this study may explain the clinical improvement often seen in narcoleptics with advancing age (8). There is a common belief that this clinical improvement is due to adaptation to the disease (reduced driving, avoidance of situations triggering cataplexy, etc.) and influenced by treatment. However, there is little literature on the evolution of narcolepsy over time. The general course of narcolepsy is hard to systematize, symptoms may be stable for several years but it may also improve or worsen (9). The severity of EDS seems to persist throughout life, even if improvements are commonly noted with age, especially after retirement. In addition, the frequency of cataplexy, hypnagogic hallucinations and sleep paralysis decreases spontaneously with age, especially for cataplexy when patients are willing to control their emotions (9). In our study, the duration of the disease could not account for all age-related changes in MSLT data (8). The less symptomatic patients on the MSLT were not diagnosed significantly later.

Considering the age-related changes in MSLT results, one may question whether the polygraphic criteria used currently to diagnose narcolepsy are appropriate. In this study, the presence of at least two SOREMPs, plus a mean sleep latency shorter than five minutes, was present in 69% of young narcoleptics but in only 50% of patients over the age of 65 (8). Using criteria such as the presence of at least one SOREMP and a mean sleep latency shorter than eight minutes would only slightly increase the sensitivity of the MSLT in the young population (from 69% to 86%) but markedly increase this sensitivity in older population (from 50% to 84%). We think that MSLT results require interpretation according to the age.

The results of this study also raise the question of whether the severity of narcolepsy decreases with advancing age (8). As an inverse correlation was found between the mean sleep latency and the number of SOREMPs on the MSLT in our population,

we may assume that the number of SOREMPs may increase with a longer duration of the nap. We could hypothesize that one or more epochs of REM sleep occurring within 20 minutes (and not 15 minutes as it was required) of the first epoch scored as sleep may increase the number of SOREMPS especially in old narcoleptics. In our study, the decrease number of SOREMPs was not associated with changes in REM sleep latency at night, a result in contrast with the classical decrease of REM sleep latency during nocturnal recordings in normal advancing aged population. These findings raise the possibility of different REM sleep triggering mechanisms involved in SOREMPs process and REM sleep latency at night.

In a normal population, MSLT scores vary with age. Several studies, but not all, reveal an enhanced sleepiness on the MSLT in elderly (10). However, sleepiness during the day is not an inevitable component of ageing, and studies of "successful aging" found normal daytime alertness on the MSLT. In narcolepsy, the disrupted sleep and REM sleep at night, which increase with age were not found to be the causal mechanism for severe daytime sleepiness (7). The interaction between sleep homeostasis and circadian rhythmicity certainly contributes to the understanding of age-related changes in the timing and quality of sleep and wakefulness states in narcolepsy (11). The major influence of age on MSLT results should therefore be taken into account when diagnosing a narcoleptic patient. However, the tendency to display SOREMPs varies greatly among narcoleptic subjects, independently of age. We may suppose that genetic factors, including the catechol-O-methyltransferase gene, are also involved (12).

References

1. Guilleminault C, Pelayo R. Narcolepsy in prepubertal children. Ann Neurol 1998; 43:135–42.
2. Guilleminault C, Mignot E, Partinen M. Narcolepsy: diagnosis and epidemiology. Controversies in the diagnosis of narcolepsy. Sleep 1994; 17:S1–S6.
3. Dauvilliers Y, Montplaisir J, Molinari N, Carlander B, Ondze B, Besset A, Billiard M. Age at onset of narcolepsy in two large populations of patients in France and Quebec. Neurology 2001; 57:2029–2033.
4. American Academy of Sleep Medicine. International Classification of Sleep Disorders, 2nd Edition, Diagnostic and Coding Manual. Westchester, Illinois. American Academy of Sleep Medicine, 2005.
5. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 1997; 20:620–629.
6. Rye DB, Dihenia B, Weissman JD, Epstein CM, Bliwise DL. Presentation of narcolepsy after 40. Neurology 1998; 50:459–465.
7. Lamphere J, Young D, Roehrs T, Wittig RM, Zorich F, Roth T. Fragmented sleep, daytime somnolence and age in narcolepsy. Clinical Electroencephalography 1989; 20:49–54.
8. Dauvilliers Y, Gosselin A, Paquet J, Touchon J, Billiard M, Montplaisir J. Effect of age on MSLT results in patients with narcolepsy-cataplexy. Neurology 2004; 62:46–50.
9. Billiard M, Besset A, Cadilhac J. The clinical and polygraphic development of narcolepsy. In: C Guilleminault, E Lugaresi (eds) Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution, New York: Raven Press, 171–185, 1983.
10. Carskadon MA, Brown ED, Dement WC. Sleep fragmentation in the elderly: relationship to daytime sleep tendency. Neurobiol Aging 1982; 3:321–327.
11. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Res Online 1998; 1:159–165.
12. Dauvilliers Y, Neidhart E, Lecendreux M, Billiard M, Tafti M. MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 2001; 6:367–372.

8
Narcolepsy in Children and Adolescents

SOŇA NEVŠÍMALOVÁ
Department of Neurology, First Faculty of Medicine, Charles University, Prague, Czech Republic

The prevalence of narcolepsy-cataplexy is, according to the latest multicentric epidemiological studies (1,2), approximately 0.05 in European as well as in North American population. Although most of the patients have had the symptoms of narcolepsy since youth, a large prospective series identified only 5% of the cases as prepubertal (3). The most current pathophysiologic model for narcolepsy involving an autoimmune-mediated destruction of hypocretin (Hcrt)/orexin-containing neurons (4) suggests the importance of early diagnosis of the disease. Some recent studies (5,6) have shown a favourable effect of autoimmune suppressive treatment using intravenous immunoglobulins in early stages of the disease, but they were open labeled trials and all positive effects were reported subjectively. Other authors have not confirmed this experience with steroid treatment (7).

Undetectable Hcrt-1 level in cerebrospinal fluid (CSF) is one of the most important diagnostic features for narcolepsy-cataplexy in children as well as adults (8,9,10). As shown in experimental studies (11), the decline of hypocretin (Hcrt) level in CSF in neurotoxically induced lesions of Hcrt neurons in rats starts very early (2–6 days after neurotoxin is applied to the lateral hypothalamus) and this effect is permanent without any recovery. A loss of 73% of Hcrt neurons causes a 50% decline in CSF hypocretin; consequently, in narcoleptic patients with undetectable CSF, virtually all of the Hcrt neurons should be lost. If the hypothesis of autoimmune role in the development of narcolepsy is accepted, the effect of immunosuppressive therapy in children with narcolepsy depends on the timing and on continuous therapeutic management as in other autoimmune diseases. Another pathophysiological possibility relates to damage done to Hcrt-containing neurons by some as yet unknown agents where autoimmune mechanisms may have only a supportive role to play. In cases with DQB1*0602 negativity, an HLA protective role against Hcrt neurons damage may be considered.

I. Clinical Presentation of Narcolepsy in Children: An Overview

Clinical diagnosis of childhood narcolepsy can be more difficult due to several atypical features compared with adults.

A. Excessive Daytime Sleepiness

Overwhelming sleep attacks in children are constant and usually of longer duration than in adults. In some patients chronic waxing and waning of drowsiness during the day with periodic superimposed sleep episodes can be observed. The children are sleepy during lessons at school and their afternoon naps can last up to two to three hours and are generally non restorative (12,13). Inattentiveness due to permanent sleepiness causes school problems including academic deterioration and social integration. Due to prolonged sleep attacks, they have less time for play as well as for homework. In young children, restlessness and motor hyperactivity can sometimes overcome the drowsiness (14) and cause behavioral problems.

B. Cataplexy

Cataplectic attacks provoked by strong emotion (most often by laughter) are reported in 80.5% of idiopathic cases (15) and they are as common as in adults. However, cataplexy may not be present during the initial years of the disease; if it is present, it can be only sporadic at first. Children tend to attach little significance to this attack. Sometimes they feel ashamed; repeated, target-oriented questioning to obtain the anamnestic data is needed. In rare cases, an isolated appearance of cataplexy preceding excessive daytime sleepiness may pose a diagnostic problem and can be misdiagnosed as astatic-myoclonic epileptic seizures (16).

C. Other Auxiliary Symptoms

- *Hypnagogic hallucinations and sleep paralysis* Hypnagogic hallucinations and sleep paralysis have been observed in 29% children presenting idiopathic narcolepsy (15). The bizarre nature of hypnagogic hallucinations and sleep paralysis may confuse children who are then too embarrassed to discuss their problems; consequently, parents must sometimes help to clarify the child's experience.
- *Confusional arousal* Unlike adults, children and adolescents suffering from narcolepsy often report sleep drunkenness (confusional arousals). Parents may experience major conflicts with the child because of confusional arousals, particularly when the children are woken-up in the early morning for school (14).
- *Automatic behavior* Automatic behavior in children suffering from narcolepsy can imitate states of cloaked consciousness of epileptic origin. A 5-year old girl from the author's study group of children threatened her younger brother with a knife during one attack of automatic behavior and this state was misinterpreted as partial seizure epilepsy.
- *Nocturnal sleep disturbances* Nocturnal sleep disturbances appearing with vivid dreams and even frequent nightmares are very frequent in children and could be misdiagnosed as parasomnias.
- *Personality changes* Narcolepsy, even in its early stages, also affects the patients' personality. Children and adolescents become more introverted, most of them with features of depression. Changes in their character comprise feeling of inferiority, sorrowfulness, emotional lability or sometimes irritability or even aggressiveness. Interpersonal conflicts are easy to arise

within the family and at school (17). Poor attention and concentration, and disciplinary problems due to sleepiness in class lead to false accusations of drug use.

- *Obesity* Obesity may occur as a co-existing problem in childhood and adolescent narcolepsy. Significant obesity is present in at least 1/4 of children suffering from narcolepsy (18,14). The tendency towards increased weight gain is manifested relatively early in the course of the disorder (19). Correlation of Hcrt and leptin metabolism can help explain the pathogenesis of this symptom (20).

In the author's series (Table 1) of 23 idiopathic cases (14 boys, 9 girls) the first symptom of the disease appeared at the mean age of 11 years (age range, 6 months – 17 years). In 12 out of 23 children their excessive daytime sleepiness was the first symptom of the disease, in 7 patients narcolepsy and cataplexy became manifested consecutively over a short period of time (several weeks up to one to two months), in 3 cases cataplexy was the first symptom and in only 1 child the disease was manifested by sleep paralysis. The diagnosis was estimated with a mean latency of 2.2 years (range, 0.3–5 years) from the first symptom. The follow-up period of the group varies between 1–20 years (mean 6.9 years). In approximately 1/2 of the cases (11 out of 23) the clinical picture covers narcolepsy and cataplexy, in more than 1/3 (7 out of 23) narcolepsy-cataplexy is combined with hypnagogic hallucination and/or sleep paralysis; 4 out of 23 children suffer from narcolepsy without cataplexy to this day, with hypnagogic hallucination or sleep paralysis or both symptoms shaping the clinical picture. Only 1 patient demonstrates the fully expressed narcoleptic tetrad (21); incidentally, he is the only case of narcolepsy-cataplexy with signal peptide mutation in Hcrt locus described in the literature (22).

Table 1 Clinical Data of Personal Observation—23 Narcoleptic Patients (14 Boys, 9 Girls)

Age at clinical diagnosis (yr)	Mean 13.8	Age range: 2–18.5[a]
Age at disease onset (yr)	Mean 11.8	Age range: 0.5–17
Latency to diagnosis (yr)	Mean 2.2	Range: 0.3–5
Follow-up period (yr)	Mean 6.9	Range: 1–20
Clinical manifestation	EDS + C	11/23
	EDS + HH and/or SP	7/23
	EDS + C + HH + SP	1/23
	EDS without C	4/23
First symptom of disease	EDS	12/23
	EDS + C	7/23
	C	3/23
	SP	1/23
MSLT findings (mean latency min ± SD)	N-C	1.7 ± 2.2 (SOREMPs 4.0 ± 1.2)
	N without C	3.9 ± 1.8 (SOREMPs 3.7 ± 1.3)

[a]Range given in years.

Abbreviations: EDS = excessive daytime sleepiness, C = cataplexy, HH = hypnagogic hallucination, SP = sleep paralysis, MSLT = multiple sleep latency test, N = narcolepsy, SOREMPs = sleep onset (REM periods).

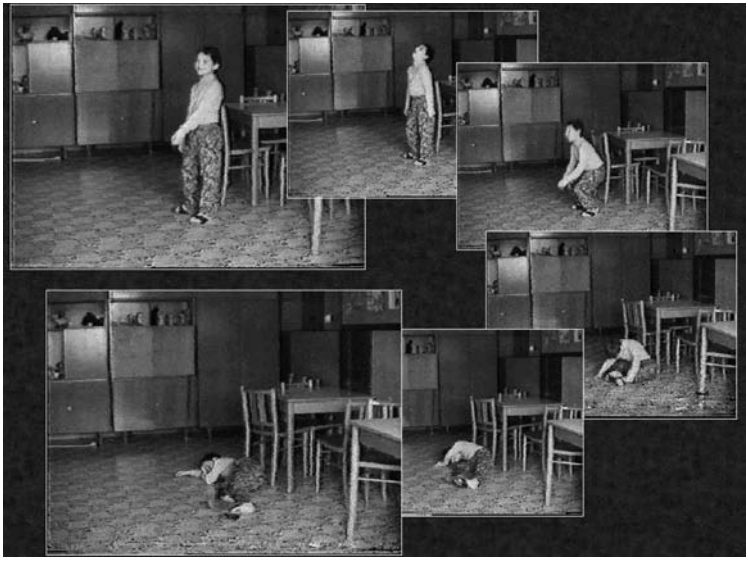


Figure 1 One of the cataplectic attacks recorded on video at the age of 9 years.

II. A Case of Hypocretin-Deficient Narcolepsy Due to a Mutation in the Hypocretin Gene

The boy's case history includes perinatal risk factors with a slightly pronounced hypotonic syndrome during infancy. Cataplectic attacks (Fig. 1), described by his parents as brief spells of head dropping provoked by laughter, first appeared at the age of 6 months. Since infancy, imperative sleep in spells of several minutes up to one hour have also been observed. He has suffered severe bulimia manifested predominantly during the night since the age of 5 years. Since puberty he has had states of hypnagogic hallucinations, sleep paralysis, automatic behaviour and behavioural disorder abnormalities. He also suffers from unquiet nocturnal sleep accompanied by slight periodic leg movements. Narcoleptic-tetrad symptoms are partially controlled with modafinil (previously methylphenidate) and fluoxetine (previously imipramine, clomipramine). He is HLA-DQBQ*0602 negative. Repeated MSLT showed extremely short latency with predominant SOREMPs. Similarly, nocturnal PSG recordings (and/or 24-h monitoring when the child was young) revealed fragmented sleep with SOREMPs. His CSF demonstrated 4 oligoclonal bands and an undetectable level of Hcrt-1. From the immunological point of view an interesting finding of thymus enlargement on computed tomography was found. Subsequent thymectomy revealed chronic inflammatory changes. Neuroimaging methods (computed tomography, magnetic resonance, position emission tomography) showed normal results.

III. Secondary Cases of Narcolepsy in Children

Secondary (symptomatic) narcolepsy-cataplexy caused by structural brain lesion is much more common in children than in adulthood involving 1/5–1/3 of all children

patients (15,13). In comparison with idiopathic cases, the age at onset in symptomatic cases is lower (6 years on average) with cataplexy as the predominant symptom. Up to 1/4 of patients described in the literature (15) have a history of status cataplecticus. The most frequent cause of symptomatic narcolepsy-cataplexy involves Niemann-Pick disease type C (15,23), brain tumors particularly in suprasellar region (24), Prader-Willi syndrome, or more rarely some other structural hypothalamic lesions caused by cerebral palsy, multiple sclerosis and/or less well-known neurological abnormalities. Careful history with neurological examination complemented by laboratory tests and neuroimaging should clarify the secondary etiology of the disease.

IV. Diagnosing Narcolepsy in Children

A clinical examination covering detailed anamnetic data and following auxiliary examinations is useful for diagnosis estimation.

A. Sleep Diary and Actigraphy

A sleep diary created for younger children by their parents and by older children themselves should illustrate the amount of daytime and nocturnal sleep and exclude the irregularity caused by inappropriate regime and poor sleep hygiene. False excessive daytime sleepiness linked to delayed (or advanced) sleep phase syndrome could be eliminated besides the sleep diary also by actigraphic recording. Actigraphy seems to be a useful screening method in cases of children's narcolepsy illustrating repeated naps during the day (12,13). In comparison with adult cases this method in childhood is better applicable owing to longer duration of sleep attacks at this period of life (Fig. 2).

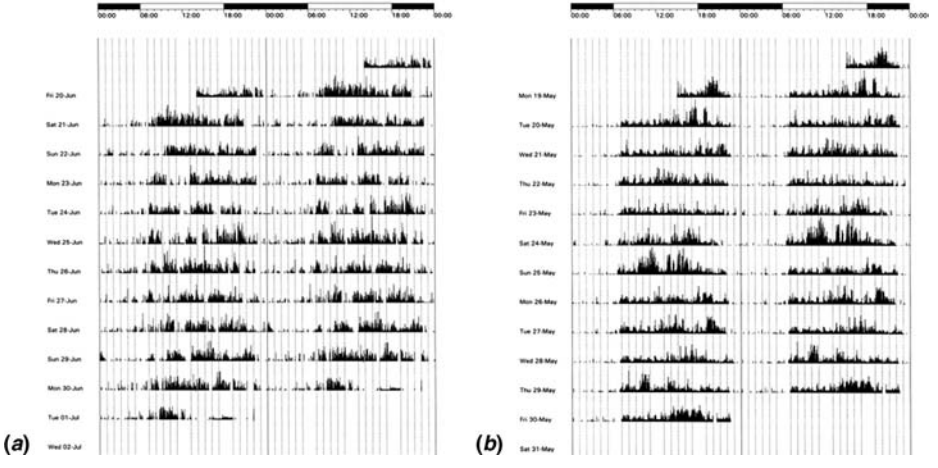


Figure 2 Actigraphic recording in a 14-year old girl with narcolepsy-cataplexy (***a***) and a control subject (***b***). Note the existence of longer periods of daytime hypoactivity illustrating daily naps (***a***) contrary to regular sleep-wake regime in the control subject (***b***).

B. Polysomnographic Studies

- *Daytime and overnight polysomnographic recordings* In toddlers and preschool children, continuous daytime records using ambulant monitoring technique can serve as a useful diagnostic method instead of multiple sleep latency test (MSLT). Long-time monitoring can exclude epileptiform discharges, which are often thought of as starting the attacks, and confirm SOREMPs accompanied by sleep attacks as an important feature of clinical diagnosis. Moreover, polygraphic monitoring can by chance detect cataplectic attacks as evidence of the narcolepsy syndrome. In symptomatic cases detailed EEG examination by neuroimaging methods is necessary to specify the diagnosis of secondary narcolepsy.

  Overnight recordings eliminate other causes of excessive daytime sleepiness such as sleep disordered breathing and/or periodic limb movements. However, their presence does not rule out the diagnosis of narcolepsy. These disorders can coexist in a significant minority of narcolepsy patients (25). Overnight polygraphic sleep records also exclude parasomnias as a cause of interrupted and unquiet nocturnal sleep.
- *Multiple sleep latency test (MSLT)* MSLT can be used in normal preschool children aged four to five years and in school-aged children. However, normal values are different in children from those in adults. Particularly adolescents can develop physiological hypersomnia (26). In preadolescents, a mean sleep latency of less than 10 minutes can be assumed as abnormal (13). In some situation children may become hypervigilant during the MSLT with marked alerting to minor stimuli, and MSLT can be invalidated by technical problems. Generally speaking, MSLT test in children requires much more patience from technicians and may sometimes require several repeats (14). Serial studies may be required in emerging childhood narcolepsy to establish a definitive diagnosis (27).

In children as well as in adults, two or more episodes of SOREMPs are considered pathological. In most prepubertal cases described in the literature (3) SOREMPs during MSLT and nocturnal recordings are fully expressed. However, in some early-stage cases the polygraphic criteria may be absent. The latency between clinical symptoms of narcolepsy and positive findings of SOREMPs in MSLT can last several months (9), and in cases with delayed development of cataplexy—according to personal observation—up to years.

C. HLA Typing

In children as well as in adults, HLA typing is a useful diagnostic tool. The presence of the DQB1*0602 haplotype makes the diagnostic probability of narcolepsy much more certain, though DQB1*0602 negativity does not exclude it. Particularly, in children with multiple familial case histories where the age of the disease onset was younger, negative HLA findings do not have any informative value (28). The absence of DQB1*0602 in young children with sporadic occurrence may also indicate a symptomatic origin of the disease. HLA examination has a predictive value in children with excessive daytime sleepiness though currently free from cataplectic attacks (13).

D. CSF Hcrt-1 Level Evaluation

As follows from relevant literature (29), the CSF level of Hcrt-1 remains stable from early infancy; hence, an undetectable level of Hcrt-1 in CSF is a very valuable diagnostic marker in children. However, some parents do not accept diagnostic lumbar puncture in their child. Estimating the Hcrt-1 level from serum would be much more considerate particularly in approaching young children, and hopefully, it will be used in the years to come. In prepubertal children undetectable Hcrt-1 level in CSF appears together with the first clinical manifestations of narcolepsy-cataplexy (9), even before polysomnographic criteria are met. Experimental data support the fact (11) that CSF Hcrt level starts declining nearly immediately after the loss of Hcrt-containing neurons in the lateral hypothalamus. How long a decreasing CSF Hcrt-1 takes to prepare the background for human narcolepsy-cataplexy to become manifest, and/or if it just joins the first symptoms of the disease should be clarified, and the outcome of such investigation could facilitate treatment management. Since the declining level of CSF Hcrt-1 is closely related to HLA positive cases (30), an even more complicated situation will appear in HLA negative narcolepsy-cataplexy cases, and/or in narcolepsy without cataplexy where CSF Hcrt-1 level is usually normal.

E. Other Auxiliary Tests

Considering the large proportion of symptomatic cases, all children with suspected narcolepsy should be examined by neuroimaging methods. Computed tomography and/or, better still magnetic resonance imaging, should be done in all preschool and early school-age children to exclude brain tumors as a cause of the disease. In children with phenotypic features of the Prader-Willi syndrome and excessive daytime sleepiness the underlying diagnosis should be clarified by detailed genetic examination. Progressive neurological impairment together with intellectual decline accompanied by cataplexy and daytime sleepiness may point to a neurometabolic background of the disorder. In such cases, Niemann-Pick disease, type C should be excluded or verified by specific enzymatic examination. Generally speaking, a high rate of cataplectic attacks, HLA negativity and detectable CSF Hcrt-1 level increase the probability of symptomatic cases. The higher the level of suspicion, the more detailed tests should be done to specify the underlying diagnosis.

V. Treatment Issues in the Pediatric Population

An autoimmune suppressive course of treatment (intravenously supplemented immunoglobulins) in the earliest stages of the disease seems to be one of the most effective possibilities (5,6), but has still to be clarified. In contrary, there is no benefit of oral steroid treatment (7).

According to generally accepted opinion (3,14,13) there is no specific treatment for narcolepsy in children in comparison with adults. The most common medications for children to counter sleepiness are modafinil, methylphenidate and pemoline; if cataplexy is a dominant symptom, clomipramine or fluoxetine are usually prescribed. Recommended dosage should be based on body weight and the initial dose should be of the lowest potency and highest efficacy. It is very important to start the treatment

as early as possible to avoid the problems at school. Stimulant (and incidentally anti-cataplectic) medication represents only one component of the treatment program.

Several non-pharmacological interventions, such as regular sleep-wake schedules and planned naps, may enhance the treatment effect. Children should be encouraged to participate in after-school and sports activities; similarly, a well-designed exercise program can have a stimulating effect. Adolescents should be counseled not to drive, use alcohol or engage in dangerous activities while drowsy. Close cooperation between teachers at school and the parents is desirable. Monitoring for emotional problems and depression and providing appropriate career counseling are important. Achieving optimal quality of life is the main target for managing childhood narcolepsy (14).

VI. Conclusion

Narcolepsy in childhood is an often under-recognised and under-diagnosed disease. Increased daytime somnolence may sometimes be the only sign for several years; the sleep attacks are of longer duration lasting up to hours, and confusional arousal with sleep drunkenness features may be present. Cataplexy may develop with delay. Some children are embarrassed to discuss their symptoms, thus adding to diagnostic difficulties. The narcoleptic tetrad is present only exceptionally. In some cases polygraphic criteria may be missing in the early stage of the disease, however, looking for HLA positivity and undetectable CSF Hcrt-1 level will greatly facilitate diagnosis. Beside the typical symptoms, some additional features including obesity and nocturnal bulimia can appear. Also poor school performance and emotional disorder are common complaints. Treatment should start as early as possible to avoid the development of problems with progress at school, and close cooperation between school and family should be maintained. In the future, childhood narcolepsy can be a key to our understanding of the pathogenesis of this disease.

References

1. Dauvilliers Y, Montplaisir J, Molinari N, et al. Age at onset of narcolepsy in two large populations of patients in France and Quebeck. Neurology 2001; 57:2029–2033.
2. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: A population-based study. Sleep 2002; 25:197–202.
3. Guilleminault C, Pelayo R. Narcolepsy in prepubertal children. Ann Neurol 1998; 43:135–142.
4. Mignot E. Sleep, sleep disorder and hypocretin (orexin). Sleep Med 2004; 5(Suppl 1):S2–S8.
5. Lecendreux M, Maret S, Bassetti C, Mouren MC, Tafti M. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old-boy. Letter to the Editor. J Sleep Res 2003; 12:1–2.
6. Dauvilliers Y, Carlander B, Rivier F, Touchon J, Tafti M. Successful management of cataplexy with intravenous immunoglobulins at narcolepsy onset. Ann Neurol 2004; 56:905–908.
7. Hecht M, Lin L, Kushida CA, Umetsu DT, Tahen S, Einen M, Mignot E. Sleep 2003; 26:809–810.
8. Tsukamoto H, Ishikawa T, Fujii Y, Fukumizu M, Sugai K, Kanbayashi T. Undetectable levels of CSF hypocretin-1 (orexin-A) in two prepubertal boys with narcolepsy. Neuropediatrics 2002; 33 :51–52
9. Kubota H, Kanbayashi T, Tanabe Y, Ito M, Takanashi J, Kohno Y, Shizumi T. Decreased cerebrospinal fluid hypocretin-1 levels near the onset of narcolepsy in 2 prepubertal children. Sleep 2003; 26:555–557.

10. Nishino S, Ripley B, Overeem S, Lammers GL, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
11. Gerashchenko D, Murillo-Rodrigues E, Lin L, Xu M, Hallet L, Nishino S. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Exp Neurol 2003; 184:1010–1016.
12. Nevšímalová S, Pretl M, Vaňková J, Blazejova K, Sonka K. Narcolepsy in children and adolescents. In: Evrard P, Richelme C, Tardieu M, eds. 3rd EPNS Congress. Bologna: Monduzzi, 1999:73–77.
13. Challamel MJ. Hypersomnia in children. In: Billiard M, ed. Sleep -Physiology, Investigation and Medicine. New York: Kluwer Academic/Plenum Publishers, 2003:457–468.
14. Wise MS. Childhood narcolepsy. Neurology 1998; 50(Suppl 1):S37–S42.
15. Challamel MJ, Mazzola ME, Nevšímalová S, Cannard C, Louis J, Revol M. Narcolepsy in children. Sleep 1994; 17(Suppl 8):S17–S20.
16. Nevšímalová S, Roth B, Zouhar A, Zemanova H. The occurrence of narcolepsy-cataplexy and periodic hypersomnia and early childhood. In: Koella WP, Obál F, Schulz H, Wisser P, eds. Sleep, 86. Stuttgart – New York. Gustav Fisher Verlag 1988:399–401.
17. Nevšímalová S, Vaňková S, Pretl M, Bruck D. Narcolepsy in children and adolescents – Clinical and psychosocial aspects (in Czech). Česka Slov Neurol Neurochir 2002; 65:169–174.
18. Dahl RE, Holttum J, Trubnick L. A clinical picture of child and adolescent narcolepsy. J Am Acad Child Adolesc Psychiatry 1994; 33:834–841.
19. Kotagal S, Krahn LE, Slocumb N. A putative link between childhood narcolepsy and obesity. Sleep Med 2004; 5:147–150.
20. Nishino S, Ripley B, Overeem S, Nevsimalova S, Lammers GL, Vankova J, Okum M, Rogers W, Brooks S, Mignot E. Low cerebrospinal fluid hypocretin (orexin) and altered energy homeostasis in human narcolepsy. Ann Neurol 2001; 50:381–388.
21. Nevšímalová S, Vaňková J, Šonka K, Faraco J, Rogers W, Overeem S, Mignot E. Hypocretin (orexin) deficiency in narcolepsy-cataplexy (In Czech). Sborn lék 2000; 101:381–386.
22. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
23. Vaňková J, Štěpánová I, Jech R, Elleder M, Ling L, Mignot E, Nishino S, Nevsimalova S. Sleep 2003; 26:427–430.
24. Marcus CL, Trescher WH, Halbower AC, Lutz J. Secondary narcolepsy in children with brain tumors. Sleep 2002; 25:435–439.
25. Kotagal S, Hartse KM, Walch JK. Characteristics of narcolepsy in preteenaged children. Pediatrics 1990; 85:205–209.
26. Carskadon M, Harvey K, Duke P, Anders TF, Litt IF, Dement WC. Pubertal change in daytime sleepiness. Sleep 1980; 3:453–460.
27. Kotagal S, Goulding PM. The laboratory assessment of daytime sleepiness in childhood. J Clin Neurophysiol 1996; 13:208–218.
28. Nevšímalová S, Mignot E, Šonka K, Arrigoni JL. Familial aspects of narcolepsy-cataplexy in the Czech Republic. Sleep 1997; 20:1021–1026.
29. Kanbayashi T, Yano T, Ishiguro H, Kawanishi K, Chiba S, Aizawa R, Sawaishi Y, Hirota K, Nishino S, Shimizu T. Hypocretin (orexin) levels in human lumbar CSF in different age groups: infants to elderly persons. Sleep 2002; 25:337–339.
30. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.

9
Idiopathic Hypersomnia

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

YVES DAUVILLIERS
CHU Montpellier, Unité de Sommeil Hôpital Gui de Chauliac, Montpellier, France

In comparison with Narcolepsy, Idiopathic Hypersomnia stands as a relatively recently identified disorder and, in contrast with it, as a disease with a less accurate clinical and biological picture. An almost pathognomonic symptom such as cataplexy is not part of the clinical features and characteristic biological markers such as sleep onset REM periods (SOREMPs), HLA-DR2/DQB1*0602 association and undetectable hypocretin-1 levels in the cerebrospinal fluid (CSF) are lacking. Finally there is no natural animal model comparable to the narcoleptic dog. This is not to say, however, that the condition should not be considered. Indeed idiopathic hypersomnia is a debilitating disease. Delineating the frontiers between narcolepsy and idiopathic hypersomnia is of definite interest for the understanding of the two conditions. Pathophysiology is still almost totally unknown.

I. Historical Background

The history of idiopathic hypersomnia goes back to the 1950s, when the symptoms of the disorder are first described in details by Roth (1). Following this initial description, Roth and his group will publish more focused articles on nocturnal sleep (2); sleep drunkenness (3); and the heredofamilial aspect in subjects with hypersomnia (4). Eventually Roth coins the term *idiopathic hypersomnia*, and distinguishes two forms of it: a monosymptomatic form characterized by excessive daytime sleepiness only, and a polysymptomatic one characterized by excessive daytime sleepiness, prolonged nocturnal sleep and major difficulty in awakening (5). At the end of the same decade idiopathic hypersomnia is referred to as *idiopathic central nervous system hypersomnia* in the Diagnostic Classification of Sleep and Arousal Disorders (6) and the distinction between the two forms unfortunately dropped out. In 1990 the International Classification of Sleep Disorders returns to the term idiopathic hypersomnia and defines it as *a disorder of presumed central nervous system cause that is associated with a normal or prolonged major sleep episode and excessive sleepiness consisting of prolonged (1–2 hours) sleep episodes of non-REM sleep* (7). The difficulty waking up in the morning is not part of the definition and the distinction between the two forms still left aside. In 1993 the upper airway resistance syndrome is first described and it is shown that a non-negligible proportion of subjects previously diagnosed with idiopathic

hypersomnia actually have upper airway resistance syndrome (8). In 1996–1998, two groups revisit idiopathic hypersomnia (9–12). They return to the initial concept of heterogeneity within idiopathic hypersomnia developed by Roth (5), with the first group describing 3 forms, a « classic » form, a « narcolepsy-like » form and a « mixed form » and the second group a polysymptomatic and a monosymptomatic forms. Eventually the second edition of the ICSD (13) distinguishes two forms of idiopathic hypersomnia, an idiopathic hypersomnia with long sleep time and an idiopathic hypersomnia without long sleep time.

II. Epidemiology

Due to the nosological uncertainty and the relative rarity of the condition, prevalence studies have not been conducted so far. A ratio of one to two patients with idiopathic hypersomnia for every ten with narcolepsy is suggested in series from sleep disorders centers (14) (Table 1). The age of onset varies from childhood to young adulthood with very few cases if any occurring after the age of 25. However, precising the age of onset short of a year is generally not possible. There is no indication of gender predominance. Family cases are frequent (4).

III. Clinical Features

Idiopathic hypersomnia with long sleep time is remarkable for three symptoms: a complaint of constant or recurrent daily excessive sleepiness and unwanted naps, longer and less irresistible than in narcolepsy, and non refreshing irrespective of their duration; night sleep is sound, uninterrupted and prolonged; morning awakening is laborious. Subjects do not awaken to the ringing of a clock, a telephone and often rely on their family members who must use vigorous and repeated procedures to wake them up. Even then, patients may remain confused, unable to react adequately to external stimuli, a state referred to as *sleep drunkenness*. Associated symptoms suggesting an autonomic nervous system dysfunction are not uncommon. They may include cold

Table 1 Number of Subjects Affected with Idiopathic Hypersomnia and Narcolepsy in Various Patient Series

Authors	Idiopathic hypersomnia (IH)	Narcolepsy (N)	Ratio IH/N (%)
Roth (1980)	167	288	57.9
Coleman et al. (1982)	150	425	35.2
Baker et al. (1986)	74	257	28.7
Aldrich (1996)	42	258	16.2
Billiard and Besset (2003)	53	380	13.9

Note: There is a declining ratio with years of subjects diagnosed with idiopathic hypersomnia due to an improved knowledge of disorders of excessive daytime sleepiness.

hands and feet, light-headedness on standing up, fainting episodes and headache that may be of migrainous type.

Idiopathic hypersomnia without long sleep time stands as isolated excessive daytime sleepiness. Daytime sleep episodes may be more irresistible and more refreshing than in idiopathic hypersomnia with long sleep time, establishing a bridge with narcolepsy without cataplexy. Abnormally long sleep or sleep drunkenness are not features of the condition.

Hypnagogic hallucinations and sleep paralysis are not exceptional with the former found occasionally, often or always in 43% of idiopathic hypersomnia subjects, and the later in 40% (10). However it has not yet been investigated whether they are more frequent in idiopathic hypersomnia with long sleep time or in idiopathic hypersomnia without long sleep time. One issue to consider is the changes in mood. In a detailed study of a group of 23 random patients with idiopathic hypersomnia, a high incidence of depressed subjects (26.1%) was reported (15). However, in another article (5) the same author stated: *against the possibility of a psychogenic origin of the affection speaks the absence of neurotic symptoms and personality factors in the majority of these patients*. Thus it is possible that in the first cited study (15) most of the depressed subjects would now be classified as having hypersomnia associated with psychiatric disorder.

Once established the disorder is stable in severity and long lasting. Spontaneous disappearance of the symptoms leads to cast doubt about the initial diagnosis. Complications are mostly encountered in social and professional functions (16).

IV. Laboratory Tests

Diagnosis is mostly clinical. However laboratory tests are indispensable to rule out some other hypersomniac conditions.

Polysomnographic monitoring of nocturnal sleep demonstrates normal sleep, except for its prolonged duration in the case of idiopathic hypersomnia with long sleep time. NREM sleep and REM sleep are in normal proportions. Sleep efficiency is commonly said to be above 90%. However in a comparative polysomnographic study of narcolepsy ($n = 174$) and idiopathic hypersomnia ($n = 37$) sleep efficiency (total sleep time per total recording time) was only 86.5 ± 2.0 in the later and sleep maintenance (total sleep time per total recording time minus latency to first sleep onset) 89.1 ± 1.8 (17). There is no sleep onset REM period. Sleep apnea syndrome and periodic limb movement disorder should theoretically be absent, but may be acceptable in the case of an early onset of idiopathic hypersomnia and of their late occurrence. Several authors have suggested the need for monitoring oesophageal pressure during sleep to rule out mild sleep-disordered breathing that may fragment sleep and induce daytime sleepiness.

The Multiple Sleep Latency Test (MSLT) demonstrates a mean sleep latency less than 10 minutes, which might be longer than in narcolepsy in the form with long sleep time and in the same range as in narcolepsy in the form without long sleep time. In the case of idiopathic hypersomnia with long sleep time MSLT seems somewhat questionable. First it may be difficult to wake the patient in preparation for the test or to keep the patient awake between naps; second, and of more concern, awakening the patient in the

morning in view of the first MSLT session precludes documenting the abnormally prolonged night sleep, and the MSLT sessions preclude recording of prolonged unrefreshing daytime sleep episode(s) of major diagnostic value.

Thus other procedures are of potential interest: 24-hour continuous polysomnography on an ad-lib sleep/wake protocol, in order to document the major sleep episode (more than 10 hours) and daytime sleep episode(s) (at least one nap of more than one hour), which still awaits standardization and validation (Fig. 1) (18), and 1-week actigraphy (19).

Association with HLA DR2/DQB1*0602 is not characteristic of idiopathic hypersomnia.

In the context of recently discovered CSF hypocretin-1 deficiency in narcolepsy with cataplexy, several groups have assessed CSF hypocretin-1. However none of the investigations done so far except one (20) has evidenced a decreased CSF hypocretin-1 level (21–23) (Fig. 2).

Computed tomography (CT) and/or magnetic resonance imaging (MRI) of the brain should be performed if there is a clinical suspicion of an underlying brain lesion.

Cognitive evoked potential (P 300) performed in the evening and in the morning, immediately after awakening and later, is of particular interest in the assessment of sleep inertia (24–25).

Psychometric/psychiatric evaluation is mandatory to exclude hypersomnia associated with a psychiatric disorder.

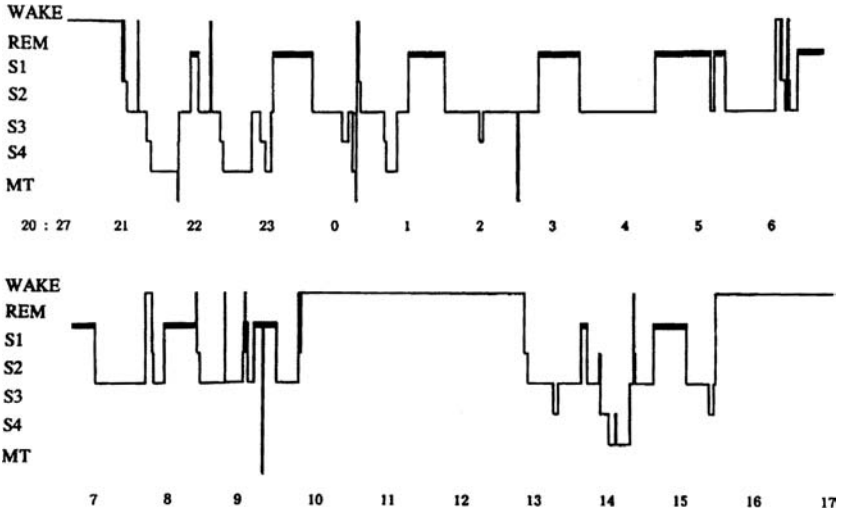


Figure 1 Continuous 24 hour polysomnography in an idiopathic hypersomnia with long sleep time subject free to turn the light on and off at will, morning and evening, and to go to bed or get up during the daytime. The duration of night sleep was 12 hours and 44 minutes and the duration of the single daytime nap 2 hours and 36 minutes, hence a total sleep time of 15 hours and 20 minutes within 24 hours.

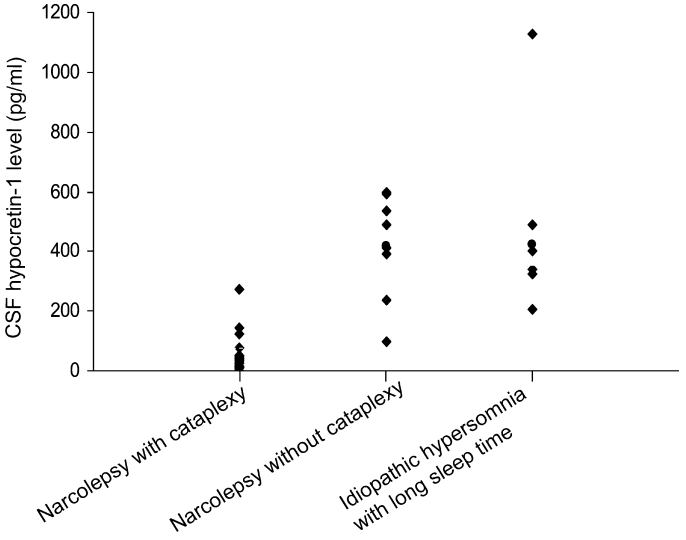


Figure 2 CSF hypocretin-1 levels in narcolepsy with cataplexy ($n = 26$), narcolepsy without cataplexy ($n = 9$) and idiopathic hypersomnia with long sleep time ($n = 7$) subjects. Each dot represents a single subject. Low CSF hypocretin-1 levels (less than 110 pg/ml) were observed in 23 out of 26 narcolepsy with cataplexy subjects. Only one out of 9 narcolepsy without cataplexy subjects had a low hypocretin-1 level. All idiopathic hypersomnia with long sleep time subjects had normal levels (subjects from Montpellier and CSF hypocretin-1 assays performed in C. Bassetti's laboratory in Zurich).

V. Frontiers

Idiopathic hypersomnia with long sleep time is obviously very different from narcolepsy with cataplexy with regard to clinical aspects, polysomnographic features, HLA typing and CSF hypocretin content. However, there may be a bridge between the two disorders through idiopathic hypersomnia without long sleep time and narcolepsy without cataplexy. Both conditions are without cataplexy, long sleep time, sleep inertia, and decreased or absent CSF hypocretin level (Table 2).

VI. Differential Diagnosis

Idiopathic hypersomnia is frequently over-diagnosed due to an unfortunate tendency to label as such hypersomnias that do not fit the criteria of either obstructive sleep apnea/hypopnea syndrome or narcolepsy.

The first diagnosis to consider is the upper airway resistance syndrome. Excessive daytime sleepiness, snoring loudly without obvious sleep apneas, and fatigue upon awakening may suggest abnormal breathing during sleep. In any case, the presence of multiple brief arousals occurring during polysomnography must draw attention

Table 2 Frontiers Between Narcolepsy with Cataplexy and Idiopathic Hypersomnia with Long Sleep Time, According to the Second Edition of the International Classification of Sleep Disorders

	Narcolepsy with cataplexy	Narcolepsy without cataplexy	Idiopathic hypersomnia without long sleep time	Idiopathic hypersomnia with long sleep time
Excessive daytime sleepiness	+	+	+	+
Cataplexy	+	−	−	−
Hypnagogic hallucinations and sleep paralysis	±	±	Not significantly present	Not significantly present
Disturbed nocturnal sleep	±	±	−	−
MSLT	3.1 ± 2.9	3.1 ± 2.9	6.2 ± 3.0	6.2 ± 3.0
SOREMPs	2 or more	2 or more	Less than 2	Less than 2
HLA DQBI*0602	Almost always	40% of subjects	As in controls	As in controls
CSF hypocretine <110 pg	90% of subjects	10–20% of subjects	As in controls	As in controls

Abbreviations: CSF, cerebrospinal fluid; MSLT, mean sleep latency test; SOREMPs, sleep onset rapid eye movement periods.
Source: From Ref. 13.

and call for monitoring oesophageal pressure in search of respiratory effort-related arousal (RERA) events (8).

Narcolepsy without cataplexy is a clinical variant of narcolepsy with cataplexy, where associated REM abnormalities such as hypnagogic hallucinations and sleep paralysis may be acknowledged and two or more SOREMPs are demonstrated on the MSLT (26). Worth mentioning is the fact that narcolepsy without cataplexy and idiopathic hypersomnia without long sleep time cannot be distinguished on a purely clinical basis.

Hypersomnia associated with psychiatric disorder, classified as "not due to a substance or known medical condition" in the second edition of the ICSD (13), should be considered in subjects with abnormal personality traits. The complaint of excessive sleepiness may be rather similar to that of patients with idiopathic hypersomnia, except that it may vary from day to day and is often associated with poor sleep at night. Polysomnographically, sleep is interrupted by frequent awakenings, REM sleep latency may be shortened, the MSLT generally does not demonstrate a short mean sleep latency. On continuous recording, it is striking that these subjects often stay in bed during the day without showing any objective sign of sleep (clinophilia) (27).

Post-traumatic hypersomnia may mimic idiopathic hypersomnia. Past medical history including an initial coma after head trauma and CT or MRI showing focal abnormalities are revealing (28–29).

Hypersomnia following a viral infection such as pneumonia, infectious mononucleosis or Guillain Barré syndrome usually develops within weeks or months after the infection, with the subject spending more and more time asleep. Prognosis is favourable but recovery may take months or years (30).

Chronic fatigue syndrome is characterized by persistent or relapsing fatigue that does not resolve with sleep or rest. The disabling fatigue is accompanied with by

joint and muscle pains, headache, poor concentration, impaired short-term memory, disturbed sleep, recurrent subjective feverish feelings and sore throat (31). Polysomnography shows reduced sleep efficiency and may include alpha intrusion into sleep EEG.

Insufficient sleep syndrome is associated with excessive daytime sleepiness, impaired concentration and lowered energy level. A detailed history of the subject's current sleep schedule is needed for the diagnosis (32).

A long sleeper is an individual who consistently sleeps more in 24 hours than the conventional amount of sleep of his or her age group. The main difference with idiopathic hypersomnia is that there is no complaint about daytime sleepiness or difficulty in awakening as long as sufficient sleep is obtained routinely to fulfil the apparent increased sleep need. If a MSLT is performed, no evidence of pathological sleepiness is present, assuming that the patient has obtained the usual sleep amount he needs for several nights prior to the procedure (7).

VII. Pathophysiology

Data are rather scant. As already mentioned, no natural model of idiopathic hypersomnia is available. However, the destruction of norepinephrine neurons of the rostral third of the locus coeruleus complex or of the norepinephrine bundle at the level of the isthmus in the cat leads to hypersomnia, with a proportional increase of NREM sleep and REM sleep suggestive of idiopathic hypersomnia. In this situation telencephalic norepinephrine is decreased and 5-HIAA and tryptophan are increased (33).

Neurochemical studies have been performed. According to one study assessing mean CSF concentrations of monoamine metabolites and using principal component analysis, all four monoamine metabolites (DOPAC, MHPG, HVA, and 5-HIAA) were highly intercorrelated in normal volunteers. In contrast HVA and DOPAC, the dopamine metabolites, did not correlate with the other two metabolites in narcoleptic subjects, and MHPG, the norepinephrine metabolite, did not correlate with the other three metabolites in idiopathic hypersomnia subjects (34). Accordingly an alteration of the dopamine system in narcolepsy and an alteration of the norepinephrine system in idiopathic hypersomnia were suggested. Much more recently, decreased CSF histamine has been found in a group of 14 subjects with idiopathic hypersomnia (35).

A dysregulation of the homeostatic and circadian process of sleep has been hypothesized (36). In line with this hypothesis a decrease in slow wave activity during the first two sleep cycles has been documented (37) as well as a higher sleep spindle density in both cerebral hemispheres at the beginning and at the end of nocturnal sleep (38). Moreover a phase delay of the melatonin and cortisol rhythms has been reported in idiopathic hypersomnia subjects, as well as a non significant longer duration of the melatonin signal (39), consistent with excessive need for prolonged nocturnal sleep, together with signs of morning sleep drunkenness. However, these results await replication.

It has been proposed that idiopathic hypersomnia represents the extreme in the distribution of habitual sleep time and that subjects with idiopathic hypersomnia may be genuine long sleepers in a permanent state of sleep deprivation (36). However

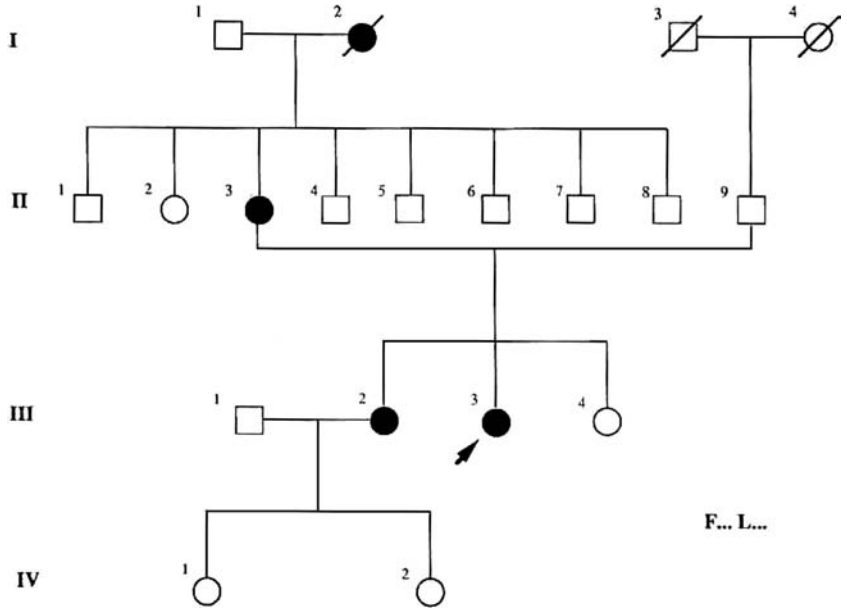


Figure 3 Pedigree of a family with an idiopathic hypersomnia with long sleep time proband and three relatives (sister, mother, and grandmother) also affected with idiopathic hypersomnia with long sleep time. In this case transmission is on the mother's side.

subjects with idiopathic hypersomnia do not report improvement of their excessive daytime sleepiness after prolonged sleeping for days (10,49).

Finally a genetic basis for idiopathic hypersomnia has been suggested (4). In our own series of 28 subjects with idiopathic hypersomnia with long sleep time, 19 (67.8%) reported to have relatives with abnormally long sleep time, difficulty in awakening and excessive daytime sleepiness (Fig. 3). However, further studies need to be performed in order to make sure that relatives affected with these symptoms do not have sleep related respiratory disorder or other cause of excessive daytime sleepiness.

VIII. Treatment

Despite a different type of excessive sleepiness in subjects with idiopathic hypersomnia and narcolepsy, the same drugs have been used in both conditions. Stimulant drugs including dextroamphetamine, methamphetamine, methylphenidate, and pemoline have been used with some success on excessive daytime sleepiness and less success on the difficulty in awakening. Unwanted effects such as headache, tachycardia or irritability have been reported. Modafinil has yielded good results in excessive daytime sleepiness (40), however the difficulty in morning awakening is not improved. To date no double blind, randomized, placebo-controlled has yet been conducted.

It is worth mentioning the occasional response to stimulating antidepressants obtained by some authors in subjects with idiopathic hypersomnia with long sleep time (10) and the shortened nocturnal sleep duration, the decreased sleep drunkenness and the relieved daytime sleepiness obtained in five out of ten idiopathic hypersomnia with long sleep time subjects treated with melatonin (2 mg of slow release melatonin administered at bedtime) (42).

Behavioral treatment possibilities are limited. Naps are of no help as they are both lengthy and non refreshing. Saturating the subjects with sleep on weekends, as recommended by Roth, does not seem to have a sustained effect (10,40).

IX. Conclusion

There is no doubt that progress has been made in the identification of idiopathic hypersomnia and the recognition of other hypersomnias previously misdiagnosed. However, idiopathic hypersomnia with long sleep time is a well-characterized clinical entity whereas idiopathic hypersomnia without long sleep time still needs clarification, especially with regard to its possible relation with narcolepsy without cataplexy. Moreover, neither HLA typing nor CSF hypocretin-1 assays have thrown light on the understanding of the pathophysiological mechanisms involved in the disorder.

References

1. Roth B. Narkolepsie a Hypersomnie s Hlediska Fysiologie Spanku, Praha, Statni Zdravonické Nakladatelstvi, 1957.
2. Rechtschaffen A, Roth B. Nocturnal sleep of hypersomniacs. Acti Nevr Sup (Praha), 1969; 11:229–233.
3. Roth B, Nevsimalova S, Rechtschaffen A. Hypersomnia with Sleep Drunkenness. Arch Gen Psychiat 1972; 26:456–462.
4. Nevsimalova-Bruhova S, Roth B. Heredofamilial aspects of narcolepsy and hypersomnia. Schweiz Neurol Neurochir Psychiat 1972; 110:45–54.
5. Roth B. Narcolepsy and hypersomnia: review and classification of 642 personally observed cases. Schweiz Arch Neurol Neurochir Psychiat 1976; 119:31–41.
6. Association of Sleep Disorders Centers. Diagnostic Classification of Sleep and Arousal Disorders, First edition, prepared by the Sleep Disorders Classification Committee, H.P. Roffwarg, Chairman. Sleep 1979; 2:1–137.
7. ICSD. International Classification of Sleep Disorders: Diagnostic and coding manual. Diagnostic Classification Steering Committee, Thorpy MJ, Chairman. Rochester, Minnesota: American Sleep Disorders Association, 1990.
8. Guilleminault C, Stoohs R, Clerk A, Cetel M, Maistros P. A cause of excessive daytime sleepiness. The upper airway resistance syndrome. Chest 1993; 104:781–787.
9. Aldrich MS. The clinical spectrum of narcolepsy and idiopathic hypersomnia. Neurology 1996; 46:393–401.
10. Bassetti C, Aldrich MS. Idiopathic hypersomnia. A series of 42 patients. Brain 1997; 120:1423–1435.
11. Billiard M. Idiopathic hypersomnia. Neurologic Clinics 1996; 14:573–582.
12. Billiard M, Merle C, Carlander B, Ondze B, Alvarez D, Besset A. Idiopathic hypersomnia. Psychiatr Clin Neurosci 1998; 52:125–129.
13. American Academy of Sleep Medicine. International classification of sleep disorders, 2nd ed. Diagnostic and Coding Manual. Westchester, Illinois: American Academy of Sleep Medicine, 2005.
14. Billiard M, Besset A. Idiopathic hypersomnia. In: Billiard M, ed. Sleep, Physiology, Investigations and Medicine. New York: Kluwer Academic/Plenum Publishers, 2003:429–435.

15. Roth B, Nevsimalova-Bruhova S. Depression in narcolepsy and hypersomnia. Schweiz Arch Neurol Neurochir Psychiat 1975; 116:291–300.
16. Broughton R, Nevsimalova S, Roth B. The socioeconomic effects (including work, education, recreation and accidents) of idiopathic hypersomnia. Sleep Res 1978; 7:217.
17. Baker TL, Guilleminault C, Nino-Murcia G, Dement WC. Comparative polysomnographic study of narcolepsy and idiopathic central nervous system hypersomnia. Sleep 1986; 9:232–242.
18. Billiard M, Dauvilliers Y. Idiopathic hypersomnia. Sleep Med Rev 2001; 5:351–360.
19. Bassetti C, Gugger M, Bischof M, Mathis J, Sturzenegger C, Werth E, Rodanov B, Ripley B, Nishino S, Mignot E. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.
20. Ebrahim IO, Sharief MK, De Lacy S, Semra YK, Howard RS, Kopelman MD, Williams AJ. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurosurgery Psychiatry 2003; 74:127–130.
21. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
22. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, Fujii Y, Nishino S, Shimizu T. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002; 11:91–93.
23. Dauvilliers Y, Baumann CR, Carlander B, Bischof M, Blatter T, Lecendreux M, Maly F, Besset A, Touchon J, Billiard M, Tafti M, Bassetti C. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry 2003; 74:1667–1673.
24. Sangal RB, Sangal JM. P300 latency: abnormal in sleep apnea with somnolence and idiopathic hypersomnia, but normal in narcolepsy. Clin Electroencephalog 1995; 26:146–153.
25. Bastuji H, Perrin F, Garcia-Larrea L. Event-related potentials during forced awakening: a tool for the study of acute sleep inertia. J Sleep Res 2003; 12:189–206.
26. Moscovitch A, Partinen M, Guilleminault C. The positive diagnosis of narcolepsy and narcolepsy's borderland. Neurology 1993; 43:55–60.
27. Billiard M, Dolenc L, Aldaz C, Ondzé B, Besset A. Hypersomnia associated with mood disorders: a new perspective. J Psychosom Res 1994; 38(suppl 1):41–47.
28. Guilleminault C, Faull KF, Miles L, van den Hoed J. Post-traumatic excessive daytime sleepiness. A review of 20 patients. Neurology 1980; 33:1584–1589.
29. Rao V, Rollings P. Sleep disturbances following traumatic brain injury. Curr Treat Opt Neurol 2002; 4:77–82.
30. Guilleminault C, Mondini S. Infectious mononucleosis and excessive daytime sleepiness. A long-term follow-up study. Arch Intern Med 1986; 146:1333–1335.
31. Fukuda D, Strauss SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann Int Med 1994; 121:953–959.
32. Aldrich M. Insufficient sleep syndrome. In: Billiard M, ed. Sleep, Physiology, Investigations and Medicine. New York: Kluwer Academic/Plenum Publishers, 2003:341–346.
33. Petitjean F, Jouvet M. Hypersomnie et augmentation de l'acide 5-hydroxy-indolacétique cérébral par lésion isthmique chez le chat. C R Hebd Séanc Acad Sci (Paris), 1970; 164:2288–2293.
34. Faull KF, Thiemann S, King RJ, Guilleminault C. Monoamine interactions in narcolepsy and hypersomnia: a preliminary report. Sleep 1986; 9:246–249.
35. Kanbayashi T, Kodama T, Kondo H, Satoh S,Miyazaki N, Kuruda K, Abe M, Nishino S, Inoue Y, Shimizu T. CSF histamine and noradrenaline contents in narcolepsy and other sleep disorders. Sleep 2004; 27:A236.
36. Billiard M, Rondouin G, Espa F, Dauvilliers Y, Besset A. Pathophysiology of idiopathic hypersomnia. Rev Neurol (Paris) 2001; 157:5S101–5S106.
37. Sforza E, Gaudreau H, Petit D, Montplaisir J. Homeostatic sleep regulation in patients with idiopathic hypersomnia. Clin Neurophysiol 2000; 11:277–282.
38. Bove A, Culebras A, Moore JT, Westlake RE. Relationship between sleep spindles and hypersomnia. Sleep 1994; 17:449–455.
39. Nevsimalova S, Blazejova K, Illnerova H, Hajek I, Vankova J, Pretl M, Sonka K. A contribution to pathophysiology of idiopathic hypersomnia. In: Ambler Z, Nevsimalova S, Kadanka Z, Rossini PM,

eds. Clinical Neurophysiology at the Beginning of the 21st Century (supplements to Clinical Neurophysiology) 2000; 53:366–370.
40. Matsunaga H. Clinical study on idiopathic CNS hypersomnolence. Jpn J Psychiatry Neurol 1987; 41:637–644.
41. Bastuji H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuropsychopharmacol Biol Psychiatry 1988; 12:695–700.
42. Montplaisir J, Fantini L. Idiopathic hypersomnia: a diagnostic dilemma. Sleep Med Rev 2001; 5: 361–362.

10
Kleine-Levin Syndrome and Other Recurrent Hypersomnias

CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

ISABELLE ARNULF
Fédération des Pathologies du Sommeil, Hôpital Pitié-Salpêtriére, Public Assistance, Hôpitaux de Paris, France

In 1925, Kleine was the first to report on a case series of 9 patients with recurrent (periodic) hypersomnia (two with increased food intake) and to propose the existence of a novel disease entity (1). Less known is the fact his case series included a young woman with menstruation-linked hypersomnia, a syndrome now considered as a distinct type of recurrent hypersomnia (2). Previous single reports with periodic hypersomnia had been published before, most notably a case by Brierre De Boismont in 1862:

> Dr. Wilson, doctor in the Middlesex hospital, observed a very remarkable case of "double-mind" in a child. Example 102—This patient was defiant, timid and modest; he ate with moderation; in his usual state, he showed by his acts that he had an honest and scrupulous nature. But, as soon as the disease reoccurred, he lost all these qualities. He slept a lot, was difficult to arouse, and as soon as he was awakened, he extemporaneously sang, recited, and acted with great ardor and aplomb. When he was not asleep, he ate ravenously. As soon as he got out of his bed, he would go close to another patient's bed, and overtly seize without any scruple, all the food he could find. Apart from this intriguing disease, he was intelligent and skillful (3).

Levin emphasized the association of periodic somnolence with morbid hunger (a symptom subsequently called megaphagia) using a single personal case and previously published reports (4). Critchley proposed the eponym Kleine-Levin syndrome (KLS) and suggested diagnostic criteria based on 26 published patients: male gender, onset in adolescence, periodic hypersomnia, compulsion to eat, and spontaneous remission (5,6).

I. Epidemiology

Using an extensive review of the PubMed indexed literature from 1962 to 2004, we found 218 Kleine-Levin cases (7). These cases were reported worldwide (Fig.1). Of note, one-sixth of these patients were reported in Israel (8), suggesting a higher vulnerability in subjects with Jewish heritage. The exact prevalence of the disease is unknown, but it is considered a very rare disease, possibly around one in a million

Figure 1 Distribution of published KLS cases in the world. Data collected through a metanalysis of the literature. *Source*: From Ref. 7.

for diagnosed cases. The exact prevalence is however likely to be underestimated especially if cases of lesser severity or without hyperphagia are more frequent than anticipated. Among patients, 68% are men, and 81% of them experience the first episode during their second decade (7). Most cases are sporadic, but a few multiplex families have been reported. Some 10% of cases occur in a pre-existent severe neurological or genetic disease background, suggesting they are secondary cases.

Menstruation-linked hypersomnia is even rarer (2). Less than 10 cases have been reported worldwide.

II. Clinical Features

KLS is characterized by recurrent episodes of dramatic hypersomnia lasting from 2 days to several weeks (median 10 days). These episodes are associated with behavioral, cognitive, eating or sexual disturbance (9), and alternate with long asymptomatic periods lasting months to years (median: 3.5 months). Minimal diagnostic criteria are reported in Table 1.

A trigger is commonly reported prior to the first episode. Triggers can include an infection in half of the cases (mostly but not exclusively viral), and much more rarely a minor head trauma, alcohol use, anesthesia, physical or emotional stress (7). The first hypersomnolent episode may be preceded by depressed mood, fatigue or headaches. The onset of hypersomnia is usually rapid, within hours or days. During attacks, patients may sleep up to 16–24 hours per day, awakening only to eat and void. Patients can be aroused but typically respond aggressively.

Mental changes, present in almost all patients, include irritability, apathy, withdrawal, slow speech, slow in answering, or on the contrary restlessness, confusion, occasionally with hallucinations. A very specific and often underappreciated

Table 1 ICSD-Revised (2005) Criteria for Recurrent Hypersomnia and KLS

A-Recurrent hypersomnia:

- The patient experiences recurrent episodes of excessive sleepiness of 2 days to 4 weeks duration
- Episodes recur at least once a year
- The patient has normal alertness, cognitive functioning and behavior between attacks
- The hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, mental disorder, medication use, or substance use disorder.

B-Kleine-Levin syndrome:

In addition to all criteria listed in A, the hypersomnia should be clearly associated with at least one of these symptoms:

- Megaphagia
- Hypersexuality
- Abnormal behavior such as irritability, aggression or odd behavior
- Cognitive abnormalities such as feeling of unreality, confusion and hallucination.

C-Menstrual associated sleep disorder:

In addition to all criteria listed in A, sleepiness occurs in association with the menstrual cycle.

symptom is a feeling of de-realization, of disconnection between mind and body, of dreamlike state, with impaired, glassy vision, or weird sensory perceptions. Two third of the patients report overeating (particularly sweets), which can lead to weight gain at the end of the episode. Overeating may present as increased appetite, compulsion to search for food or as a simple passive increase in food intake when food is presented to the subject. Some patients on the contrary loose appetite and need to be awakened and encouraged to eat.

Hypersexuality, with inadequate sexual advances, increased masturbation and de-inhibited behavior, is observed in 43% of the cases. It is twice more frequent in boys than in girls. Decreased or flattened mood, increased anxiety, compulsion to pace, write or sing, and a generally childish, regressive behavior are possible. Some patients report a depressed mood (with rare cases of suicidal attempt), anxiety, or feeling of frustration during the episodes. Polydipsia, water retention, increased sweating and a reddish face flushing are occasionally observed (10). Episodes often stop abruptly with a typical short lasting bout of insomnia (e.g., a single night) and hypomania. A partial amnesia of the episode is often present. Physical and psychiatric examinations are unremarkable in KLS patients between attacks. Persisting cognitive and behavioral abnormalities have, however, rarely been reported (11,12).

Relapses may occur at intervals of a few days to up to 20 years, although over time patients exhibit a decrease in duration, frequency and severity of episodes (8). However, the median duration of the disease calculated from published meta-analyzed data using Kaplan-Meier method, is 8 years, longer than often assumed.

In menstruation-linked periodic hypersomnia, episodes are short lasting (a few days) and occur mostly before (rarely during or after) every menstruation (2). Associated behavioral disturbances include withdrawal, apathy, and sometimes brief visual hallucinations, but no megaphagia or de-inhibited behavior.

III. Pathophysiology

The pathophysiology of KLS is unknown. The clinical picture and a few hormonal studies, documenting a disturbed hypothalamic-pituitary axis in some (13–15), but by far not all patients (16), suggest an hypothalamic dysfunction. A moderate and transient decrease in hypocretin-1 levels in the cerebrospinal fluid has been recently described (17). However, the wide range of clinical signs and abnormalities observed on EEG and functional SPECT imagery suggest also a variable dysfunction of other brain areas (mainly frontal, temporal (11), and possibly thalamic (18) area). Nothing is known regarding the pathophysiology of menstruation associated hypersomnia but the sexual hormone axis is functioning normally (2).

IV. Etiology

The cause of all recurrent hypersomnia (menstruation-linked recurrent hypersomnia and KLS) is unknown. Postmortem examinations have revealed inconsistent findings in 4 KLS cases. In a few cases, a focal encephalitis may be responsible (19,20) but cerebrospinal fluid analysis is typically normal. Traumatic or vascular brain lesions have been implicated in rare cases (21,22). The clinical and polygraphic features

of KLS can be similar to those found in hypersomnolent depression (atypical depression) and idiopathic hypersomnia; this includes a possible positive response to lithium (23). A recent study found increased Human Leukocyte Antigen (HLA)-DQB1*0201 allele frequency in KLS versus controls (24). This finding, together with the typically young age of onset, the remittent-relapsing nature of the disease course, and the possible infectious trigger may suggest an auto-immune or an infectious mediation.

A. Diagnosis

Minimal criteria for periodic hypersomnia, KLS type or menstruation-linked periodic hypersomnia type are described in Table 1. Episodes should last from two days to four weeks (although longer episodes may occur in some patients) and should reoccur at least once a year; a minimum of two episodes is needed. In KLS, the hypersomnia must be associated with either cognitive, behavioral, eating or sexual disturbance. In atypical/incomplete forms of KLS, symptomatic and psychiatric forms of hypersomnia should be excluded first.

EEG recordings are useful to confirm the diagnosis and to rule out epilepsy. In KLS, the EEG is abnormal in as many as 70% of the KLS patients during episodes. It shows a slowing of the background activity, sometimes associated with bursts of generalized, moderate to high voltage 5–7 Hz waves, but no epileptic activity (25). The brain MRI is typically normal. Polysomnographic findings in KLS are variable and highly dependant on the delay between the onset of the episode and the laboratory test. They include a normal or decreased sleep efficiency, a normal architecture, an increase or decrease of slow-wave sleep, and sleep onset REM episodes (SOREMPs). Prolonged (e.g., 24-hour) polysomnographic (or actigraphic) monitoring can document an increased amount of sleep per day (16). Multiple Sleep Latency Test may draw normal results or decreased sleep latency with SOREMPs in one fourth of the cases (25,26). The patients may however have difficulties to comply with the test procedure.

In menstruation-linked periodic hypersomnia, the background EEG rhythm may also be slowed during episodes, as reported in most KLS patients.

B. Differential Diagnosis

The most important differential diagnoses for KLS are epilepsy and psychiatric conditions. These include depression, bipolar mood disorders, and psychogenic/somatoform hypersomnia (27,28), with the caveat that many true KLS patients are also often misdiagnosed as psychotic, depressed or hysterical patients. Less common causes of recurrent hypersomnia are seen in association with brain neoplasia (craniopharyngioma, third and fourth ventricle, hypothalamic tumor, pinealoma), encephalitis, and benzodiazepine/endozepine intoxications (recurrent stupor) (29). Medical causes of recurrent confusion including epilepsy and those in the context of alcohol abuse should also be kept in mind. In the rare Kluver-Bucy syndrome, patients with bi-temporal lesions experience increased sexuality and oral exploration, but with no recurrent aspect (30). Patients with Lewy Body dementia may also have major fluctuation of alertness, associated with confusion, hallucination and behavior disturbances,

but they are older than KLS patients, have a permanent cognitive defect and frequently parkinsonism.

V. Treatment

Cases of KLS are few and treatment is not well codified. Most therapeutic attempts are ineffective or effective in only a subset of cases. The unpredictable nature of the relapses and the tendency of the syndrome to spontaneous resolve make it difficult to systematically assess drug efficacy. During hypersomnolent episodes, stimulants can be tried, usually with limited symptomatic effects. Lithium (31–33) but rarely other mood stabilizers antiepileptic drugs (34) have been found to prevent the recurrence of further episodes in isolated cases.

The treatment of menstruation-linked periodic hypersomnia is generally the complete suppression of menstruation (2).

References

1. Kleine W. Periodische Schlafsucht. Monatsschrift fur Psychiatrie und Neurologie 1925; 57:285–320.
2. Billiard M, Guilleminault C, Dement WC. A menstruation-linked periodic hypersomnia. Kleine-Levin syndrome or new clinical entity? Neurology 1975; 25(5):436–443.
3. Brierre de Boismont A. Des hallucinations ou histoire raisonnée des apparitions, des visions, des songes, de l'extase, des rêves, du magnétisme et du somnambulisme. Paris: Germer Baillière, 1862.
4. Levin M. Periodic somnolence and morbid hunger: a new syndrome. Brain 1936; 59:494–504.
5. Critchley M. Periodic hypersomnia and megaphagia in adolescent males. Brain 1962; 85:627–656.
6. Critchley M, Hoffman H. The syndrome of periodic somnolence and morbic hunger (Kleine-Levin syndrome). BMJ 1942; 1:137–139.
7. Arnulf I, Zeitzer J, File J, Farber N, Mignot E. Kleine-Levin syndrome: a meta-analysis of 186 cases in the literature. 2005; Submitted.
8. Gadoth N, Kesler A, Vainstein G, Peled R, Lavie P. Clinical and polysomnographic characteristics of 34 patients with Kleine-Levin syndrome. J Sleep Res 2001; 10(4):337–341.
9. American Academy of Sleep Medicine. The international Classification of Sleep Disorders - Revised. Chicago, IL: In press, 2005.
10. Hegarty A, Merriam AE. Autonomic events in Kleine-Levin syndrome. Am J Psychiatry 1990; 147(7):951–952.
11. Landtblom AM, Dige N, Schwerdt K, Safstrom P, Granerus G. Short-term memory dysfunction in Kleine-Levin syndrome. Acta Neurol Scand 2003; 108(5):363–367.
12. Sagar RS, Khandelwal SK, Gupta S. Interepisodic morbidity in Kleine-Levin syndrome. Br J Psychiatry 1990; 157:139–141.
13. Fernandez JM, Lara I, Gila L, O'Neill of Tyrone A, Tovar J, Gimeno A. Disturbed hypothalamic-pituitary axis in idiopathic recurring hypersomnia syndrome. Acta Neurol Scand 1990; 82(6):361–363.
14. Chesson AL Jr, Levine SN, Kong LS, Lee SC. Neuroendocrine evaluation in Kleine-Levin syndrome: evidence of reduced dopaminergic tone during periods of hypersomnolence. Sleep 1991; 14(3): 226–232.
15. Malhotra S, Das MK, Gupta N, Muralidharan R. A clinical study of Kleine-Levin syndrome with evidence for hypothalamic-pituitary axis dysfunction. Biol Psychiatry 1997; 42(4):299–301.
16. Mayer G, Leonhard E, Krieg J, Meier-Ewert K. Endocrinological and polysomnographic findings in Kleine-Levin syndrome: no evidence for hypothalamic and circadian dysfunction. Sleep 1998; 21(3):278–284.
17. Dauvilliers Y, Baumann CR, Carlander B, et al. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry 2003; 74(12):1667–1673.

18. Huang Y, Guilleminault C, Kao P, Liu F. SPECT findings in Kleine-Levin syndrome. Sleep 2005; http://www.journalsleep.org/accepted.asp.
19. Merriam AE. Kleine-Levin syndrome following acute viral encephalitis. Biol Psychiatry 1986; 21(13):1301–1304.
20. Carpenter S, Yassa R, Ochs R. A pathologic basis for Kleine-Levin syndrome. Arch Neurol 1982; 39(1):25–28.
21. Drake ME Jr. Kleine-Levin syndrome after multiple cerebral infarctions. Psychosomatics 1987; 28(6):329–330.
22. Kostic VS, Stefanova E, Svetel M, Kozic D. A variant of the Kleine-Levin syndrome following head trauma. Behav Neurol 1998; 11(2):105–108.
23. Bassetti C, Aldrich M. Idiopathic hypersomnia. A series of 42 patients. Brain 1997; 120(8):1423–1435.
24. Dauvilliers Y, Mayer G, Lecendreux M, et al. Kleine-Levin syndrome: an autoimmune hypothesis based on clinical and genetic analyses. Neurology 2002; 59(11):1739–1745.
25. Reynolds CF, 3rd, Black RS, Coble P, Holzer B, Kupfer DJ. Similarities in EEG sleep findings for Kleine-Levin syndrome and unipolar depression. Am J Psychiatry 1980; 137(1):116–118.
26. Rosenow F, Kotagal P, Cohen BH, Green C, Wyllie E. Multiple sleep latency test and polysomnography in diagnosing Kleine-Levin syndrome and periodic hypersomnia. J Clin Neurophysiol 2000; 17(5): 519–522.
27. Godenne G. Report of a case of recurrent hysterical pseudostupors. Journal of Mental Disorders 1966; 141:670–677.
28. Jeffries J, Lefebvre A. Depression and mania associated with Kleine-Levin-Critchley syndrome. Canadian Psychiatric Association Journal 1973; 18:439–444.
29. Lugaresi E, Montagna P, Tinuper P, et al. Endozepine stupor. Recurring stupor linked to endozepine-4 accumulation. Brain 1998; 121(Pt 1):127–133.
30. Terzian H, Dalle-Ore P. Syndrome of Kluver and Bucy reproduced in man by bilateral removal of the temporal lobes. Neurology 1955; 5:373–380.
31. Poppe M, Friebel D, Reuner U, Todt H, Koch R, Heubner G. The Kleine-Levin syndrome - effects of treatment with lithium. Neuropediatrics 2003; 34(3):113–119.
32. Smolik P, Roth B. Kleine-Levin syndrome ethiopathogenesis and treatment. Acta Univ Carol Med Monogr 1988; 128:5–94.
33. Kellett J. Lithium prophylaxis of periodic hypersomnia. Br J Psychiatry 1977; 130:312–316.
34. Mukaddes NM, Kora ME, Bilge S. Carbamazepine for Kleine-Levin syndrome. J Am Acad Child Adolesc Psychiatry 1999; 38(7):791–792.

11
Spectrum of Narcolepsy

CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

I. Introduction

Westphal in 1877 and Gélineau in 1880, the first describers of narcolepsy, correctly identified excessive daytime sleepiness and cataplexy as the essential features of the disorder (1,2). At the beginning of the 20th century, Löwenfeld, Henneberg, Redlich and Adie were among the first to accept narcolepsy as a "disease sui generis" (3–5) (6). Until the 1930s and even later other authors including Blocq and Wilson considered however narcolepsy a nonspecific form of severe hypersomnia due to different potential causes (7,8).

Following the publication of large patients series by Daniels, Redlich, Wilder and Yoss and Daly, narcolepsy was finally accepted as a distinct disorder characterized by the presence in most patients of two key-symptoms (excessive daytime sleepiness (EDS), cataplexy) and in about half of the patients of "REM sleep phenomena" (sleep paralysis, hallucinations) (9–12) (6). However, only 20% to 30% of the patients exhibit Yoss and Daly's "narcoleptic tetrade," proving the existence of a variable clinical spectrum of narcolepsy, including narcolepsy without cataplexy (12–15). The reports of severe, "overwhelming" EDS, cataplexy-like episodes, sleep paralysis and hallucinations also by patients with non-narcoleptic EDS (Table 1) and even by normal subjects stress on the other hand the lack of specificity (with the exception of definite, severe cataplexy) of narcoleptic symptoms for narcolepsy (14–18).

The discovery of the association between sleep onset REM periods (SOREMPs) and narcolepsy, initiated discussions about REM and NREM phenomena as well as REM and NREM variants of narcolepsy (19–25). Similarly, the discovery of other biological markers of the disease such as specific HLA markers (26) and low cerebrospinal fluid levels of hypocretin-1 (27), was soon followed by reports of "HLA-negative" narcolepsy and narcolepsy with normal CSF hypocretin-1 levels (17,27–29). In addition, recent studies have shown that SOREMPs and HLA positivity are linked and can be found also in patients with non-narcoleptic EDS and even normal individuals (30–33).

In the absence of a golden standard for the diagnosis of narcolepsy, the spectrum of this disorder remains a matter of debate (14–16,34–36). This chapter will review our knowledge about the borderland of narcolepsy, a better understanding of which is crucial to advance our knowledge on the pathophysiology and treatment of narcolepsy.

Table 1 Clinical Symptomatology and Results of Ancillary Tests in Patients with Narcolepsy with Cataplexy ($n = 39$), Monosymptomatic Narcolepsy ($n = 28$), and Idiopathic Hypersomnia ($n = 42$)

Parameter	Idiopathic hypersomnia	Mono-symptomatic narcolepsy	Narcolepsy with cataplexy
Subjects (n)	42	28	39
Mean age at diagnosis (years)	35	34	46
Female : male	27:15	13:15	25:14
Caucasian : African American : Asian	39:3:0	20:7:1	27:11:1
Automatic behavior	61%	25–35%	31–35%
Sleep paralysis	40%	27–36%	49–58%
Hypnagogic hallucinations	43%	14–30%	74–75%
Hours of sleep per weekday	8.4 ± 1.9	7.7 ± 0.4	7.8 ± 0.3
Time to get going in the morning (min)	42	48	36
Sleep efficiency	93 ± 5%	93 ± 5%	86 ± 12%
Total sleep time (min)	464 ± 50	468 ± 47	432 ± 75
Number of awakenings (>1 min)	20 ± 11	9 ± 7	17 ± 10
Slow wave sleep (% of total sleep)	8 ± 5	8 ± 4	5 ± 4
REM sleep (% of total sleep)	18 ± 7	19 ± 4	16 ± 6
Mean sleep latency on MSLT	4.3 ± 2.1	2.8 ± 1.1	2.2 ± 1.2
Periods of sleep onset with REM/ chances on polysomnogram + MSLT	12/303 (4%)	93/188 (49%)	106/210 (50%)
DR2 (number of positives/total tested)	6/18 (33%)	4/7 (57%)	9/11 (82%)
DQ1 (number of positives/total tested)	13/18 (72%)	7/7 (100%)	9/10 (90%)

Source: From Ref. 47.

II. Narcolepsy Without Cataplexy

In most series not more than 10% to 20% of patients are given the diagnosis of narcolepsy in the absence of a history of cataplexy (so called monosymptomatic narcolepsy). This diagnosis is difficult and such causes of excessive daytime sleepiness (EDS) as subtle form of sleep apnea-hypopnea syndromes and chronic sleep insufficiency syndrome may be misdiagnosed as monosymptomatic narcolepsy. Although the existence of monosymptomatic narcolepsy has been questioned, its existence is suggested by three lines of evidence.

First, in patients with narcolepsy, the first appearance of EDS and cataplexy can be separated by several years. Cataplexy usually appears concomitantly or within a few years from the onset of EDS, but in rare cases it follows EDS by an interval of up to 40 to 50 years (12,13,15,37,38).

Second, in patients with the full narcoleptic tetrade the frequency and severity of the different symptoms can vary considerably. For example, cataplexy can occur one hundred times daily or only a few times in a lifetime and vary from a mild feeling of

weakness which is unnoticeable by others to severe atonic attacks with falls to the ground. Furthermore, cataplexy typically decreases in severity after its appearance (not uncommonly already in the first five years) and may in fact even disappear. Hence, depending on the severity and frequency criteria chosen for the diagnosis of cataplexy, patients with EDS may be diagnosed as having narcolepsy with or without cataplexy.

Third, patients with narcolepsy with cataplexy often have a positive family history of imperative (narcolepsy-like) EDS but rarely of cataplexy, a phenomenon that also suggests the existence of monosymptomatic (milder, incomplete) forms of narcolepsy (39–41). This hypothesis is supported by the observation of longer mean sleep latencies on multiple sleep latency test (MSLT) in patients without cataplexy and/detectable CSF hpocretin when compared with narcoleptics with definite ("clear cut," "true") cataplexy and/or CSF hypocretin deficiency (15,29,42). Monosymptomatic narcolepsy may indeed be viewed as a variant of narcolepsy with cataplexy due to insufficient genetic and/or environmental components.

Narcolepsy without cataplexy is a diagnosis of exclusion. The following criteria may be used for its recognition: (*i*) overwhelming ("imperative") EDS with daily or almost daily napping/"sleep attacks," for at least three months, starting in the second or third decade; (*ii*) sleep latency $\leq 5-8$ minutes and ≥ 2 SOREMPs on MSLT; (*iii*) no evidence by history, clinical findings or ancillary tests for other causes of EDS such as sleep apnea-hypopnea syndromes, chronic sleep insufficiency/irregular sleep-wake habits, EDS associated with psychiatric disease, substance abuse, head trauma (43). Three findings can give further support to the diagnosis of narcolepsy without cataplexy: (*i*) history of sleep paralysis, hallucinations or familial narcolepsy; (*ii*) low CSF hypocretin-1 levels; (*iii*) DQB1*0602 positivity.

The existence of a NREM-variant of narcolepsy without cataplexy, that is a narcolepsy form without SOREMPs on (repeated) MSLT, is controversial and, if at all, probably rare ($<5\%$ of all cases of narcolepsy). Follow-up studies in narcoleptics that develop or lose clinical or polygraphic REM phenomena over time give support to the existence of such a "NREM narcolepsy" (38,44). Many of these patients are probably diagnosed as having a (narcolepsy-like or monosymptomatic) variant of idiopathic hypersomnia (16).

A DQB1*0602 positivity was reported to be as low as 41% of patients with narcolepsy without cataplexy, and 55% to 85% of those with atypical or mild cataplexy (45). A recent review of published data as essentialy confirmed these percentages (see below). Our group reported a DQB1*0602 positivity in 14 (89%) out of 16 narcoleptics with nondefinite (that is, mild, rare, or atypical) cataplexy, an in eight out of nine narcoleptics without cataplexy (15,46). These observations may be linked with our reluctance in making the diagnosis of narcolepsy without cataplexy before other potential causes of EDS have not been ruled out.

Low/absent CSF hypocretin-1 levels were found in 17% of the 113 patients with narcolepsy without cataplexy reported in the literature (reviewed in Ref. 47). In a series of nine narcoleptics without cataplexy we found normal levels in eight patients and low (but detectable) levels in one patient (46). In a series of nine patients with narcolepsy without cataplexy, Krahn et al. reported normal CSF hypocretin-1 levels in all patients (48).

Narcolepsy without cataplexy, idiopathic hypersomnia and EDS associated with psychiatric disorders share clinical features (young age, depressed mood, neurovegetative symptoms) that make their differentiation arduous (16–18). Because of this diagnostic uncertainty, regular follow-ups are crucial in patients with a diagnosis of narcolepsy without cataplexy.

III. Other Forms of Monosymptomatic Narcolepsy/Isolated Cataplexy

In about 10% of patients with narcolepsy, cataplexy precedes the onset of EDS usually by several months and rarely by a few years, however as much as 28 years has been reported (13). Isolated cataplectic attacks are typical of childhood narcolepsy and, particularly when frequent, should always evoke the possibility of a symptomatic form of the disease (49).

A few cases of familial and isolated cataplexy have been reported in the literature (50,51). Cataplexy without EDS may be accompanied by normal sleep latencies but sleep onset REM periods (SOREM) on the MSLT (Bassetti and Vella, unpublished personal observation).

Rarely narcolepsy first manifest with attacks of isolated sleep paralysis with or without hallucinations. EDS and cataplexy usually follow within months or a few years. Sleep paralysis, particularly when occurring frequently and at sleep onset and when associated with EDS should evoke the possibility of (monosymptomatic) narcolepsy, although other sleep disorders should be considered in the differential diagnosis (52).

IV. Familial Narcolepsy/Narcolepsy in Twins

A. Familial Narcolepsy

Familial forms of narcolepsy with cataplexy, first reported by Westphal (1), are rare and probably represent only 1% to 2% of all cases of narcolepsy, even if some authors have reported percentages as high as 4% to 10% (40,53–55). Even less common are families with more than two affected individuals (multiplex families) (39,41).

Clinical, neurophysiological and HLA features of familial narcolepsy have been reported by several authors (40,41,55–57). No significant differences were found in clinical and neurophysiological findings between familial and sporadic forms of narcolepsy (55). Conversely, HLA-DQB1*0602 positivity is less common in familial narcolepsy, being found in only about 50% of patients with both narcolepsy and cataplexy (see Chapter).

A family with both multiple sclerosis and narcolepsy affecting four different generations was described by Yoss and Daly (39). Other families with both multiple sclerosis and narcolepsy were described by Ekbom and by Nevsimalova et al. (40,58). Schrader et al. reported the occurence of both narcolepsy with cataplexy and multiple sclerosis in a twin pair (59). A family with narcolepsy associated with deafness, autosomal dominant ataxia was recently described (60). Familial forms of sleep paralysis and cataplexy with and without other narcoleptic symptoms (EDS, sleep paralysis) are exceptional (50–52,61).

In a study of 23 patients with narcolepsy with cataplexy from nine different families Mignot et al. found that 12 (60%) out of 20 patients had normal CSF hypocretin-1 levels, and 9 (41%) out of 22 patients were HLA-DQB1*0602 negative (29). Interestingly, all nine HLA negative patients were found to have normal CSF hypocretin-1 levels.

B. Narcolepsy in Twins

Twenty monozygotic narcoleptic twin pairs have been reported in the literature (41,57,62–65). Thirteen (65%) out of these 20 pairs are discordant for narcolepsy. Unfortunately, clinical, neurophysiological, and HLA findings in most reported twin pairs are scarse or lacking [the first 16 pairs were reviewed by Mignot (57)].

Sixteen (89%) out of the 18 twin pairs tested were HLA DQB1*062 positive. Conversely, two twin pairs were HLA DQB1*062 negative. In one HLA DQB1*0602 positive and concordant pair CSF hypocretin-1 levels were normal in both twin (65). In a second discordant HLA DQB1*0602 positive pair CSF hypocretin-1 levels were low only in the affected twin.

V. HLA-Negative Narcolepsy and Narcolepsy with Normal CSF Hypocretin-1 Levels

A. HLA-Negative Narcolepsy

In a review of published data the HLA haplotype DQB1*0602 was found in 87% of 574 patients with narcolepsy and typical (definite, clear cut) cataplexy, in 44% of 117 patients with narcolepsy and atypical cataplexy, in 33% of patients with narcolepsy, SOREMPs but without cataplexy, and in 25% of 1416 controls (Chapter).

B. Narcolepsy with Normal CSF Hypocretin-1 Levels

Low/absent CSF hypocretin-1 levels are the most sensitive ancillary test for the diagnosis of narcolepsy with cataplexy. In a recent review of published data of 150 HLA-positive patients with narcolepsy and cataplexy low and absent CSF hypocretin-1 levels were found in 93% and 66% of patients, respectively (Fig. 1). A similar sensitivity has been

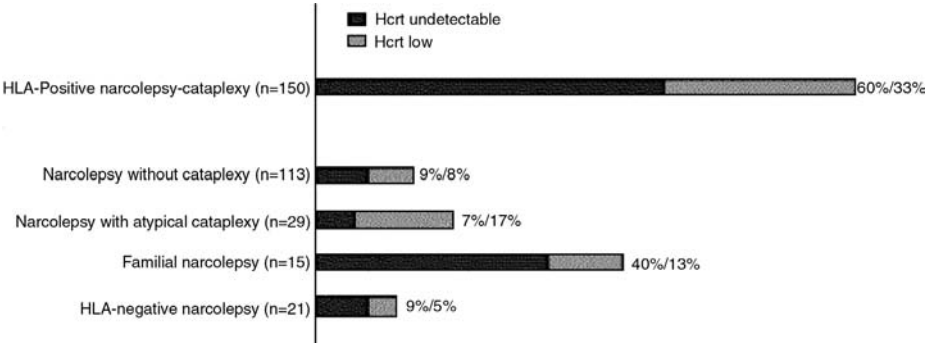


Figure 1 CSF hypocretin-1 levels in patients with narcolepsy with cataplexy and other forms of narcolepsy. *Source*: From Ref. 47.

reported in the series of Mignot et al. [87% of 101 patients (29)], Dauvilliers et al. [88% of 26 patients (46)], and Krahn et al. [71% of 17 patients (48)]. It should be noted, however, that the presence of a definite ("true," "clear cut") cataplexy was only in the series of Mignot et al. and Baumann and Bassetti, who used identical criteria (66). As mentioned above, in fact, mild, atypical or rare cataplexy usually heralds the presence of normal CSF hypocretin-1 levels.

Nevertheless, patients with definite cataplexy and normal CSF hypocretin-1 levels also exist. A significant percentage of these patients are HLA-negative and have a positive family history for EDS or narcolepsy. In a series of 65 patients with narcolepsy and normal hypocretin-1 levels. Mignot et al. found that 25 (38%) had a typical cataplexy, 17 (26%) a positive family history, and 41 (66%) a negative HLA-DQB1*0602 typing (29).

VI. Symptomatic Narcolepsy

Narcolepsy-like syndromes have been reported in association with stroke, encephalitis, hypothalamic disorders, brain tumor, multiple sclerosis (and other autoimmune diseases), endocrine disorders, neurodegenerative disorders (e.g., Norrie's disease, Möbious syndrome, Niemann-Pick disease type C, Coffy-Lowry syndrome) and head trauma. Occasionally, symptomatic narcolepsy (as well as cataplexy) may resolve with specific treatment of the underlying condition (67).

Many of these reports, particularly the oldest ones (68), are questionable/uncertain. In some cases of narcolepsy following mild traumatic brain injury, for example, it is likely that head trauma triggered an underlying condition rather than being per se the cause of narcolepsy (69). On the other hand, cataplexy-like episodes appearing for in association for example with neurodegenerative disorders or brainstem lesions often appear to differ in terms of senso-motor manifestations, triggering factors, duration or accompanying features from cataplexy in patients with idiopathic narcolepsy.

Symptomatic narcolepsy appears to arise from brain lesions of different topography, although posterior hypothalamic (70–72) and brainstem (17,73,74) are more often involved (75).

An HLA-DQB1*0602 positivity was found in (only) 47% of 59 patients with symptomatic narcolepsy reported in the literature (75).

In a review of the literature published in 2005 a total of 66 patients with symptomatic EDS and/or narcolepsy was found, in whom CSF hypocretin-1 assessments were available (75). In most patients absent/low CSF hypocretin-1 levels were found[a] (29,72,75–78). In most of these patients, however, cataplexy was unclear or not present. Conversely, in a few patients with symptomatic narcolepsy and definite cataplexy (e.g., in an HLA-DQB1*0602 negative patient with an isolated demyelinating lesion in the dorsal pons, Fig. 2) (17); in an HLA-DQB1*0602 positive patient with Niemann-Pick disease type C (29) normal CSF hypocretin-1 levels were found.

Hence, HLA positivity and CSF hypocretin-1 deficiency are predictive of cataplexy in idiopathic but not in symptomatic narcolepsy.

---

[a]It is likley that a publication bias may be involved in this high frequency.

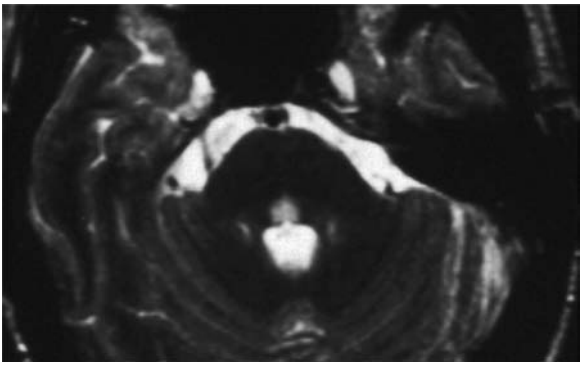


Figure 2 Symptomatic narcolepsy due to a monophasic, acute pontine tegmental demyelination (EDS, cataplexy, hallucinations, sleep paralysis, REM sleep behavior, SOREMPs) with normal CSF hypocretin-1 levels in an HLA DQB1*0602 negative patient. *Source*: From Ref. 17.

VII. "Psychiatric" Narcolepsy

Several papers in the 1920–1950s hypothesized a psychiatric origin of narcolepsy (79–81). There are indeed multiple observations that link narcolepsy with psychiatry.

First, psychogenic stress may trigger the onset of narcolepsy. Orellana et al. analysed the life events in the year preceding the onset of narcolepsy and found psychologic distress as one of the triggering factors (82). We have personally observed the acute onset of hypocretin-deficient narcolepsy in a 40-year old man who for the first time in his life had to take care of his four children in the absence of his wife.

Second, psychiatric symptoms are common in narcolepsy. In up to one third of patients, a psychiatric diagnosis is considered first (13). Depression, anxiety, reduced self-esteem have been found in up to 20% to 40% of patients (83). This high frequency is in parts related to the high psychosocial burden of narcolepsy (84). In addition, psychiatric disorders may reflect a primary brain dysfunction in narcolepsy.

Third, hallucinatory syndromes suggestive of schizophrenia have been described in narcoleptics (85,86). Typically, these hallucinations are not improved by antipsychotics but can be controlled by stimulants (85). Less commonly, psychosis is secondary to stimulant treatment (1–3% of patients on longterm treatment). The co-occurrence of narcolepsy and schizophrenia is also possible although rare [0.5–9 cases in a population of 1 million (86)].

Fourth, narcolepsy-like symptoms have been reported in patients with psychiatric symptoms and disorders. Rosenthal reported the presence hallucinations and sleep paralysis-like episodes associated with anxiety/fear (so-called halluzinatorisch-kataplektisches Angstsyndrom) in about 25% of 70 patients with schizophrenia (87). Cataplexy-like ("pseudocataplexy") and sleep paralysis-like episodes ("pseudo-sleep paralysis") have been observed also in patients with other psychiatric disorders (88,89). In the general population, hypnagogic/hypnopompic hallucinations and sleep paralysis are also associated with anxiety, depressive, psychotic symptoms (90–92). Transient cataplexy-like episodes have been recently reported after

discontinuation of venlafaxine in two HLA-negative depressive/bipolar patients with normal CSF hypocretin-1 levels (93). Noteworthy, we have observed the occurence of both "true" cataplexy and cataplexy-like spells in a hypocretin-deficient narcoleptic patient, in analogy to the existence of "true" seizures and pseudoseizures in epileptic patients (unpublished observation). A similar observation was made by Krahn et al. in a patient with diagnosis of definite narcolepsy but no assessment of hypocretin-1 levels (89). The existence of such a psychogenic modulation is further supported by the observation of a placebo effect in a recent trial, in which cataplexy was treated with sodium oxybate (94).

VIII. Summary and Conclusions

Clinically, the spectrum of narcolepsy includes at one end "classical narcolepsy" characterized by severe excessive daytime sleepiness, "true" ("definite", "clear cut") cataplexy and, in about 20% to 30% of cases, the full tetrade (Table 2). At the other end of the disorder milder/incomplete forms such as narcolepsy without cataplexy and other variants of the so-called monosymptomatic narcolepsy can be observed. Furthermore, single narcolepsy-like manifestations (severe EDS, cataplexy, sleep paralysis, hallucinations, short mean sleep latencies, and SOREMPs on MSLT) can be observed also in other sleep, neurological and psychiatric disorders as well as in normal subjects.

Pathophysiologically this spectrum reflects the recruitment of similar "final common pathways" through multiple genetic and environmental factors (Table 2). The presence of HLA-positivity, "definite" cataplexy and absent/low CSF hypocretin-1 levels are linked and characterize "classical" and severe forms of sporadic narcolepsy. Familial, symptomatic and rare sporadic forms of narcolepsy do not necessarily imply the presence of HLA-positivity and hypocretin deficiency and seem to arise from other environmental and genetic factors.

Table 2 The Clinical and Pathophysiological Spectrum of Narcolepsy

Clinical spectrum
Narcolepsy-like manifestations[a] in normal subjects:
Sleep disorders
Neurological disorders
Psychiatric disorders
Narcolepsy with mild/atypical cataplexy and other forms of monosymptomatic narcolepsy
Narcolepsy with definite cataplexy
Narcoleptic tetrade
Pathopyhsiological spectrum
Sporadic narcolepsy (usually HLA positive and with CSF hypocretin-1 deficiency)
Familial narcolepsy
Symptomatic narcolepsy (usually in the course of brain lesions)
Psychiatric narcolepsy

[a]EDS, cataplexy-like episodes, sleep paralysis, hallucinations, short mean sleep latency, and SOREMPs on MSLT.

The old Adie-Wilson debate (narcolepsy as disease "sui generis" versus the existence of different forms of narcolepsy, "the narcolepsies") has returned in actuality (5,8). More research is now needed to identify the role of specific environmental agents, non-HLA genetic factors (such as monoamine oxydase-A (MAO-A), tumor necrosis alpha (TNF-α), catachol-O methyltransferase) (COMT) (95), neurotransmitter dysfunctions other than hypocretin deficiency (96), and psychogenic factors in the etiology and manifestations of narcolepsy and its spectrum.

References

1. Westphal C. Eigentümliche mit Einschlafen verbundene Anfälle. Archiv für Psychiatrie und Nervenkrankheiten 1877; 7:631–635.
2. Gélineau JBE. De la narcolepsie. Gazette des Hôpitaux 1880; 53, 54:626–628, 35–37.
3. Löwenfeld L. Ueber Narkolepsie. Münchner Medizinische Wochenschrift 1902; 49:1041–1045.
4. Henneberg R. Ueber genuine Narkolepsie. Neurol Zbl 1916; 30:282–290.
5. Adie WJ. Idiopathic narcolepsy: a disease sui generis: with remarks on the mechanism of sleep. Brain 1926; 49:257–306.
6. Redlich E. Ueber Narkolepsie. Zeitschrift für die gesamte Neurologie und Psychiatrie 1924; 95:256–270.
7. Blocq P. Semeiology of sleep. Brain 1891; 14:112–126.
8. Wilson SA. The narcolepsies. Brain 1928; 51:63–109.
9. Redlich E. Epilegomena zur Narkolepsiefrage. Zeitschrift für Gesundheit Psychiatrie und Neurologie 1931; 136:129–173.
10. Daniels LE. Narcolepsy. Medicine 1934; 13:1–122.
11. Wilder J, ed. Narkolepsie. Berlin: Springer, 1935.
12. Yoss RE, Daly DD. Criteria for the diagnosis of the narcoleptic syndrome. Mayo Clinic Proceedings 1957; 32:320–328.
13. Parkes JD, Baraitser M, Marsden CD, Asselman P. Natural history, symptoms and treatment of the narcoleptic syndrome. Acta Neurologica Scandinavica 1975; 52(5):337–353.
14. Aldrich M. The clinical spectrum of narcolepsy and idiopathic hypersomnia. Neurology 1996; 46:393–401.
15. Sturzenegger C, Bassetti C. The clinical spectrum of narcolepsy with cataplexy: A reappraisal. J Sleep Res 2004; 13:395–406.
16. Bassetti C, Aldrich M. Idiopathic Hypersomnia. A study of 42 patients. Brain 1997; 120:1423–1435.
17. Bassetti C, Gugger M, Bischof M, et al. The narcoleptic borderland: A multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.
18. Bassetti CL. Idiopathic hypersomnia. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. Philadelphia: Elsevier Saunders; 2005.
19. Vogel G. Studies in psychophysiology of dreams III. The dreams of narcolepsy. Archives of general psychiatry 1960; 3:421–428.
20. Rechtschaffen A, Wolpert W, Dement W, Mitchell S, Fischer C. Nocturnal sleep of narcoleptics. Electroencephalography and Clinical Neurophysiology 1963; 15:599–609.
21. Passouant P, Schwab RS, Cadilhac J, Baldy-Moulinier M. Narcolepsie-Cataplexie. Etude du sommeil de nuit et du sommeil de jour. Revue Neurologique 1964; 111:415–426.
22. Dement W, Rechtschaffen A, Gulevich G. The nature of the narcoleptic sleep attack. Neurology 1966; 16:18–33.
23. Berti Ceroni G, Coccagna G, Gambi D, Lugaresi E. Considerazioni clinico-poligrafiche sulla narcolessia essenziale "a sonno lento." Sistema Nervoso 1967; 2:81–89.
24. Hishikawa Y, Nanno H, Tachibana M, Furuya E, Koida H, Kaneko Z. The nature of sleep attack and other symptoms of narcolepsy. Electroencephalography and Clinical Neurophysiology 1968; 24.
25. Roth B, Bruhovà S, Lehovskù M. REM sleep and NREM sleep in narcolepsy and hypersomnia. Electroencephalography and Clinical Neurophysiology 1969; 26:176–182.
26. Honda Y, Asaka A, Tanaka Y, Juji T. Discrimination of narcolepsy by using genetic markers and HLA. Sleep Research 1983; 12:254.

27. Nishino N, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
28. Nishino S, Ripley B, Overeem S, et al. Low cerebrospinal fluid hypocretin (orexin) and altered energy homeostasis in human narcolepsy. Ann Neurol 2001; 50:381–388.
29. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
30. Bishop C, Rosenthal L, Helmus T, Roehrs T, Roth T. The frequency of multiple sleep onset REM periods among subjects with no excessive daytime sleepiness. Sleep 1996; 19(9):727–730.
31. Chervin RD, Aldrich M. Specificity of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Neurology 1995; 45 (Suppl 4):A432–A433 (abstract).
32. Mignot E, Young T, Lin L, Finn L, Palta M. Reduction of REM sleep latency associated with HLA-DQB1*0602 in normal adults. Lancet 1998; 351:727.
33. Mignot E, Lin L, Finn L, et al. Correlates of sleep-onset REM periods during the Multiple Sleep Latency Test in community adults. Brain 2006; (in press).
34. Roth B. Narkolepsie und Hypersomnie. In: Roth B, ed. Narkolepsie und Hypersomnie. Berlin: VEB Verlag; 1962.
35. Roth B. Narcolepsy and hypersomnia. Basel: Karger Libri; 1980.
36. Moscowitch A, Partinen M, Guilleminault C. The positive diagnosis of narcolepsy and narcolepsy's borderland. Neurology 1993; 43:55–60.
37. Yoss RE, Daly DD. Narcolepsy. Medical Clinics of North America 1960; 44:955–968.
38. Billiard M, Besset A, Cadilhac J. The clinical and polygraphic development of narcolepsy. In: Guilleminault C, Lugaresi E, eds. Sleep/Wake disorders: Natural history, epidemiology, and long-term evolution. New York: Raven Press; 1983:171–185.
39. Daly DD, Yoss RE. A family with narcolepsy. Mayo Clinic Proceedings 1959; 34:313–319.
40. Nevsimalova S, Mignot E, Sonka K, Arrigoni JL. Familial aspects of narcolepsy-cataplexy in the Czech republic. Sleep 1997; 20:1021–1026.
41. Baumann CR, Dauvilliers Y, Maly FE, Mignot E, Bassetti CL. Normal CSF-1 (orexin-A) levels in dementia with diffuse Lewy bodies. Eur Neurol 2004; (in press).
42. Baumann C, Khatami R, Werth E, Bassetti CL. Hypocretin (orexin) deficiency predicts severe objective excessive daytime sleepiness in narcolepsy with cataplexy. J Neurol Neurosurg Psychiatry 2006; 77:402–404.
43. American Academy of Sleep M. The International Classification of Sleep Disorders. Second Edition ed; 2005.
44. Guilleminault C, Mignot E, Partinen M. Controversies in the diagnosis of narcolepsy. Sleep 1994; 17:S1–S6.
45. Mignot E, Hajduk R, Black J, Grumet FC, Guilleminault C. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. Sleep 1997; 20(11):1012–1020.
46. Dauvilliers Y, Baumann CR, Maly FE, Billiard M, Bassetti C. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, other hypersomnias and neurological conditions. Journal of Neurology, Neurosurgery and Psychiatry 2003; 74(1667–1673).
47. Baumann C, Bassetti CL. Hypocretin and narcolepsy. Lancet Neurology 2005; 10:673–682.
48. Krahn LE, Pankratz VS, Olivier L, Boeve BF, Silber M. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: Relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25(7):733–736.
49. Challamel MJ, Mazzola ME, Nevsimalova S, Cannard C, Louis J, Revol M. Narcolepsy in children. Sleep 1994; 17:17–20.
50. Gelardi JAM, Brown JW. Hereditary cataplexy. Journal of Neurology, Neurosurgery, and Psychiatry 1967; 30:455–457.
51. Hartse KM, Zorick F, Sicklesteel J, Roth T. Isolated cataplexy: A familial study. Henry Ford Hosp Med J 1988; 36:24–27.
52. Roth B, Bruhova S, Berkova L. Familial sleep paralysis. Schweizer Archiv für Neurologie und Psychiatrie 1968; 102:321–330.
53. Nevsimalova-Bruhova S, Roth B. Heredofamilial aspects of narcolepsy and hypersomnia. Schweizer Archiv für Neurologie und Psychiatrie 1972; 110:45–54.
54. Guilleminault C, Mignot E, Grumet FC. Familial patterns of narcolepsy. Lancet 1989; 335:1376–1379.
55. Billiard M, Pasquié-Magnetto V, Heckmann M, et al. Family studies in narcolepsy. Sleep 1994; 17:S54–S59.

56. Mayer G, Lattermann A, Mueller-Echardt G, Sbanborg E, Meier-Ewert K. Segregation of HLA genes in multicase narcolepsy families. J Sleep Res 1998; 7:127–133.
57. Mignot E. Genetic and familial aspects of narcolepsy. Neurology 1998; 50 (Suppl 1):S16–S22.
58. Ekbom K. Familial multiple sclerosis associated with narcolepsy. Archives of Neurology 1966; 15:337–344.
59. Schrader H, Gotlibsen OB, Skomedal GN. Multiple sclerosis and narcolepsy/cataplexy in a monozygotic twin. Neurology 1980; 30(1):105–108.
60. Melberg A, Hetta J, Dahl N, Nennesmo I, et al. Autosomal dominant cerebellar ataxia deafness and narcolepsy. Journal of Neurological Sciences 1995; 134:119–129.
61. Dahlitz M, Parkes JD. Sleep paralysis. Lancet 1993; 341:406–407.
62. Partinen M, Hublin C, Kaprio J, Koskenvuo M, Guilleminault C. Twin studies in narcolepsy. Sleep 1994; 17:S13–S16.
63. Kaprio J, Hublin C, Partinen M, Heikkilä K, Koskenvuo M. Narcolepsy-like symptoms among adult twins. Journal of Sleep Research 1996; 5(1):55–60.
64. Honda M, Honda Y, Uchida S, Miyazaki S, Tokunaga K. Monozygotic twins incompletely concordant for narcolepsy. Biol Psychiatry 2001; 49:943–947.
65. Khatami R, Maret S, Werth E, et al. A monozygotic twin pair concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 363:1199–1200.
66. Anic-Labat S, Guilleminault C, Kraemer HC, Meehan J, Arrigoni J, Mignot E. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. Sleep 1999; 22:77–87.
67. Onofrij M, Curatola L, Ferracci F, Fulgente T. Narcolepsy associated with primary temporal lobe B-cells lymphoma in a HLA DR2 negative subject. Journal of Neurology, Neurosurgery, and Psychiatry 1992; 55:852–853.
68. Bonduelle M, Degos C. Symptomatic narcolepsies: a critical study. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy Proceedings of the First International Symposium on Narcolepsy. Montpellier, France: Spectrum Publications, Inc.; 1976:313–332.
69. Lankford DA, Wellmann JJ, O'Hara C. Posttraumatic narcolepsy in mild to moderate closed head injury. Sleep 1994; 17:25–28.
70. Schwartz WJ, Stakes JW, Hobson JA. Transient cataplexy after removal of a craniopharyngioma. Neurology 1984; 34:1372–1375.
71. Aldrich MS, Naylor MW. Narcolepsy associated with lesions of the diencephalon. Neurology 1989; 39:1505–1508.
72. Gledhill RF, Bartel PR, Yoshida Y, Nishino H, Scammell T. Narcolepsy caused by acute disseminated encephalomyelitis. Arch Neurol 2004; 61(5):758–760.
73. Stahl SM, Layzer RB, Aminoff MJ, Townsend JJ, Feldon S. Continuous cataplexy in a patient with a midbrain tumor: The limp man syndrome. Neurology 1980; 30:1115–1118.
74. D'Cruz OF, Vaughn BV, Gold SH, Greenwood RS. Symptomatic cataplexy in pontomedullary lesions. Neurology 1994; 44:2189–2191.
75. Nishino S, Kanbayashi T. Symptomatic narcolepsy, cataplexy, and hypersomnia, and their implications in the hypothalamic hypocretin/orexin system. Sleep Med Rev 2005; 9:269–310.
76. Scammell TE, Nishino S, Mignot E, Saper CB. Narcolepsy and low CSF orexin (hypocretin) concentration after stroke. Neurology 2001; 56:1751–1753.
77. Dempsey OJ, McGeoch P, de Silva RN, Douglas NJ. Acquired narcolepsy in an acromegalic patient who underwent pituitary irradiation. Neurology 2003; 61:537–540.
78. Lagrange AH, Blaivas M, Gomez-Hassan D, Malow BA. Ramussen's syndrome and new-onset narcolepsy, cataplexy, and epilepsy in an adult. Epilepsy and behavior 2003; 4:788–792.
79. Willey MM. Sleep as an escape mechanism. Psychoanalytic review 1924; 11:181–183.
80. Missriegler A. On the psychogenesis of narcolepsy. Journal of Nervous and Mental Diseases 1941; 93:141–162.
81. Pai MN. Hypersomnia syndromes. British Medical Journal 1950; 1:522–524.
82. Orellana C, Villemin E, Tafti M, Carlander B, Besset A, Billiard M. Life events in the year preceding the onset of narcolepsy. Sleep 1994; 17:S50–S53.
83. Roth B, Nevsimalova S. Depression in narcolepsy and hypersomnia. Schweizer Archiv für Neurologie und Psychiatrie 1975; 116:291–300.
84. Goswami M. The influence of clinical symptoms on quality of life in patients with narcolepsy. Neurology 1998; 50(Suppl 1):S31–S36.

85. Douglass AB, Hays P, Pazderka F, Russell JM. Florid refractory schizophrenias that turn out to be treatable variants of HLA-associated narcolepsy. Journal of Nervous and Mental Diseases 1991; 179:12–17.
86. Kishi Y, Konishi S, Koizumi S, et al. Schizophrenia and narcolepsy: A review with a case report. Psychiatry and Clinical Neurosciences 2004; 58:117–124.
87. Rosenthal C. Ueber das Auftreten von halluzinatorisch-kataplektischen Angstsyndrom, Wachanfällen und ähnlichen Störungen bei Schizophrenen. Monatschrift für Psychiatrie und Neurologie 1939; 102:11–38.
88. Bassetti C, Aldrich M. Narcolepsy. Neurologic Clinics of North America 1996; 14:545–571.
89. Krahn LE, Hansen MR, Shepard JW. Pseudocataplexy, Psychosomatics 2001; 42:356–358.
90. Ohayon M, Priest RG, Caulet M, Guilleminault C. Hypnagogic and hypnopompic hallucinations: pathological phenomena. British Journal of Psychiatry 1996; 169:459–467.
91. Ohayon MM, Zulley J, Guilleminault C, Smirne S. Prevalence and pathologic associations of sleep paralysis in the general population. Neurology 1999; 52(6):1194–1200.
92. Ohayon M. Prevalence of hallucinations and their pathological associations in the general population. Psychiatry Research 2000; 97.
93. Nissen C, Feige B, Nofzinger EA, et al. Transient narcolepsy-cataplexy syndrome after discontinuation of the antidepressant venlafaxine. J Sleep Res 2005; 14(2):207–208.
94. US Xyrem Multicenter Study G. A randomized, double-blind, placebo-controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. Sleep 2002; 25(1):42–49.
95. Maret S, Tafti M. Genetics of narcolepsy and other major sleep disorders. Swiss Med Wkly 2005; 135:662–665.
96. Bassetti C. Selective hypocretin (orexin) neuronal loss and multiple signaling deficiencies. Neurology 2005; 65:1152–1153.

12
Sleep Paralysis, State Transition Disruptions, and Narcolepsy

JAMES ALLAN CHEYNE
Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada

Sleep paralysis (SP) is a brief period of paralysis associated with REM occurring at sleep onset (hypnagogic) or offset (hypnopompic) (1,2). Descriptions and explanations of SP experiences have appeared in the scholarly, medical and scientific literature for at least 1500 years, often under the rubric of nightmare (3) and, more recently, as part of the narcoleptic tetrad/pentad (1). The nature of the relation between SP and narcolepsy is a focus of the second part of the present chapter.

As for many sleep- and dream-related experiences it was only in the last half of the last century that developments in the neurophysiology of sleep and dreams opened up the possibility of providing thoroughgoing accounts not only of the contexts and conditions of SP (3), but also of the puzzling and terrifying hallucinations that accompany SP. In addition, however, a comprehensive understanding of SP requires the development of a phenomenological taxonomy of SP. Although there has been no shortage of narrative accounts, only recently have empirical studies attempted to develop a systematic taxonomy that includes a conceptual structure compatible with known REM neurophysiology.

I. A Brief Neurophenomenology of Sleep Paralysis Experiences

In a series of studies employing a growing multinational web-based archival data base (4), we have repeatedly found that the majority of SP experiences can be described by a meaningful factor structure consisting of what we have called Intruder, Incubus, and Vestibular-Motor (V-M) experiences (Table 1) (3–5). Intruder experiences incorporate delusions of a threatening presence plus visual, auditory, and tactile hallucinations consistent with the perception of a human or supernatural agent. Incubus experiences appear to be directly attributable to REM-induced motor paralysis and consequent breathing problems, often experienced as suffocation, restraint, and pressure (typically on the chest). When accompanied by Intruder experiences these sensations are often interpreted as assault. V-M hallucinations, on the other hand, are all clearly related to location and movement (linear and rotational) of the bodily-self. This factor structure has emerged for frequency, intensity, and spatial characteristics of SP hallucinoid

Table 1 Varimax-Rotated Factor Structure of Isolated Sleep Paralysis (ISP) and for Sleep Paralysis with Narcolepsy, Night Waking, or Sleep Attacks

	ISP[a]				SP with narcolepsy, night waking, sleep attacks[b]			
	Intruder	Incubus	Accelerative	Vestibular-Motor	Intruder	Incubus	Accelerative	Vestibular-Motor
Sensed presence	0.76	0.11	0.01	0.11	0.77	0.09	0.01	0.11
Visual	0.69	0.08	0.02	0.20	0.68	0.08	0.05	0.20
Tactile	0.65	0.28	0.07	0.03	0.66	0.31	0.13	0.02
Auditory	0.57	0.05	0.17	0.13	0.60	0.03	0.12	0.18
Bed covers	0.53	0.13	0.14	0.01	0.54	0.16	0.18	0.01
Breathing	0.07	0.76	0.07	0.03	0.14	0.74	0.03	0.12
Suffocation	0.15	0.65	0.09	0.04	0.14	0.67	0.19	−0.02
Death thoughts	0.03	0.62	0.02	0.18	0.03	0.63	−0.03	0.25
Pressure	0.37	0.58	0.07	0.02	0.37	0.54	0.06	0.08
Pain	0.12	0.51	0.10	0.11	0.08	0.54	0.23	0.06
Elevator	0.12	0.11	0.76	0.15	0.17	0.10	0.76	0.14
Falling	0.07	0.15	0.72	0.10	0.09	0.14	0.70	0.13
Spinning	0.11	0.07	0.64	0.08	0.06	0.14	0.67	0.10
Flying	0.08	0.02	0.55	0.48	0.12	0.01	0.59	0.46
Out-of body	0.10	0.11	0.21	0.80	0.11	0.14	0.13	0.79
Autoscopy	0.06	0.13	0.00	0.79	0.06	0.15	0.15	0.76
Floating	0.15	0.01	0.49	0.59	0.18	0.03	0.03	0.57
Moving	0.20	0.11	0.14	0.44	0.25	0.14	0.14	0.51
% Variance	13.16	12.13	12.23	12.14	13.90	12.34	13.11	12.22

[a]N = 10224.
[b]N = 2847.

experiences. It should be noted, however, that SP experiences are seldom as narratively elaborate as conventional dreams or as the labels for these factors might suggest. Indeed, often SP experiences are isolated and uninterpreted sensations. Nonetheless, they continue to be organized as the same factor structure.

The patterning and generality of results suggests to us that these factors reflect low-level neural mechanisms, corresponding strikingly to expectations based on REM neurophysiology. That Intruder and Incubus experiences entail features of threat and assault implicates amygdalar functioning. Specifically, the function of an amygdalar threat activated vigilance system (4) is to disambiguate incipient warning signs of danger through lowered sensory thresholds and selective perceptual biases (6). The amygdalae are normally activated by external threat cues, but can be activated during REM (7), and, in interaction with REM-generated imagery, are hypothesized to produce hallucinations of threat and assault (4,5). V-M hallucinations, on the other hand, appear to be associated with REM activation of brainstem, cerebellar and cortical vestibular centers. One factor, accelerative, consists of sensations of linear and angular acceleration (e.g., flying and spinning) and the other, V-M, appears to involve floating sensations and out-of-body experiences. The patterning of V-M hallucinations is consistent with the hypothesis of a REM-based activation of an integrated bodily-self-neuromatrix (4). These neurological hypotheses require further assessment using neuroimaging studies, which will, unfortunately be quite challenging given the unpredictable nature of SP episodes.

This taxonomy provides both qualitative and quantitative measures of SP experiences with corresponding neurophysiological interpretations. The availability of a systematic taxonomy can potentially enable a more intensive examination of relations between isolated SP (ISP) and SP accompanied by additional sleep-related problems, such as narcolepsy and related sleep-wake state transition instabilities.

II. Narcolepsy, State-Transition Disruption, and Severity of Sleep Paralysis

Although SP and associated hallucinations have long been considered as a part of the narcoleptic tetrad/pentad along with cataplexy, daytime sleepiness, and sometimes disrupted sleep, recent evidence of high prevalence of SP in the general population would appear to call this inclusion into question. It is important, however, to consider, in addition to prevalence, the relative severity of episodes. Two conceptually independent measures of SP severity are the frequency of SP (1,8) and the intensity of hallucinations (4) accompanying SP episodes. In the remainder of this chapter, we present evidence of similarities and differences in frequency and intensity of SP episodes among individuals reporting or not reporting narcolepsy diagnosis as well as more general state-transition disruptions.

All participants in our surveys indicate, via check-boxes, if they have received a diagnosis of narcolepsy, cataplexy, sleep attacks, or night waking. To validate these reports, a subset of 919 SP respondents also completed a sleep questionnaire including a web version of the Epworth Sleepiness Scale several months later. SP respondents not reporting narcolepsy were well within the normal range (mean = 8.00; SD = 4.29),

Table 2 Means and Significant Differences in Age and Age of Onset for Respondents Not Reporting or Reporting Narcolepsy, with or Without Cataplexy[a]

	Narcolepsy with cataplexy	Narcolepsy without cataplexy	No narcolepsy	F[b]	*p*
Age	35.41 (12.46)	32.39 (11.55)	28.99 (10.29)	18.28	.001
Age of onset	18.44 (11.01)	17.19 (9.72)	16.59 (8.18)	1.75	.173
N	149	54	12868	—	—

[a]SD in parentheses.
[b]df = 1, 13067.

whereas those reporting narcolepsy scored significantly higher (mean = 13.07; SD = 5.54; $t(13) = 17.51$; $p < .0001$).

Respondents reporting sleep attacks also obtained higher Epworth scores (mean = 11.60; SD = 4.78; $t(44) = 44.04$; $p < .0001$). Other follow-up questions determined that respondents who had previously reported night waking reported twice the frequency of waking (mean = 3.00; SD = 2.14) than respondents not reporting night waking problems (mean = 1.50; SD = 1.31); $t(135) = 7.04$; $p < .0001$). Thus, there is evidence of stability over time of sleep disruption reports. Respondents are also asked to estimate the frequency, intensity, and age of onset of their SP episodes. For the present analyses, our original 6-point frequency scale was converted to three ICSD severity categories to facilitate comparison with a major epidemiological study of SP (8). Intensity of SP experiences is rated on a 7-point Likert scale.

The percentages of SP experients reporting a diagnosis of narcolepsy without (1.6%) or with cataplexy (0.4%) is an order of magnitude greater than the highest estimates of prevalence in the general population (1), yet constitute a small fraction of all SP experients in the sample. Those reporting narcolepsy, night waking, and sleep attacks were slightly older but reported similar ages of SP onset (Tables 2 and 3). The distribution of estimated onset age for SP with narcolepsy was less leptokurtic (Fig. 1) (Kurtosis = 1.25; S.E. = .34) than for those reporting isolated SP (ISP, i.e., without narcolepsy, sleep attacks, and night waking) (Kurtosis = 1.86; S.E. = .04). Although the difference was not significant for narcolepsy, it was significant for

Table 3 Means and Significant Differences in Age and Age of Onset for Respondents Not Reporting or Reporting Sleep Attacks and Night Waking[a]

	Night waking	No night waking	F[b]	*p*
Age	31.77 (11.03)	28.44 (10.05)	208.41	.001
Age of onset	16.22 (9.07)	16.70 (8.00)	6.76	.009
N	2423	10648	—	—
	Sleep attacks	No sleep attacks	F[b]	*p*
Age	32.93 (11.85)	28.84 (10.19)	102.90	.001
Age of onset	16.97 (9.57)	16.59 (8.12)	1.43	.232
N	686	12385	—	—

[a]SD in parentheses.
[b]df = 1, 13067.

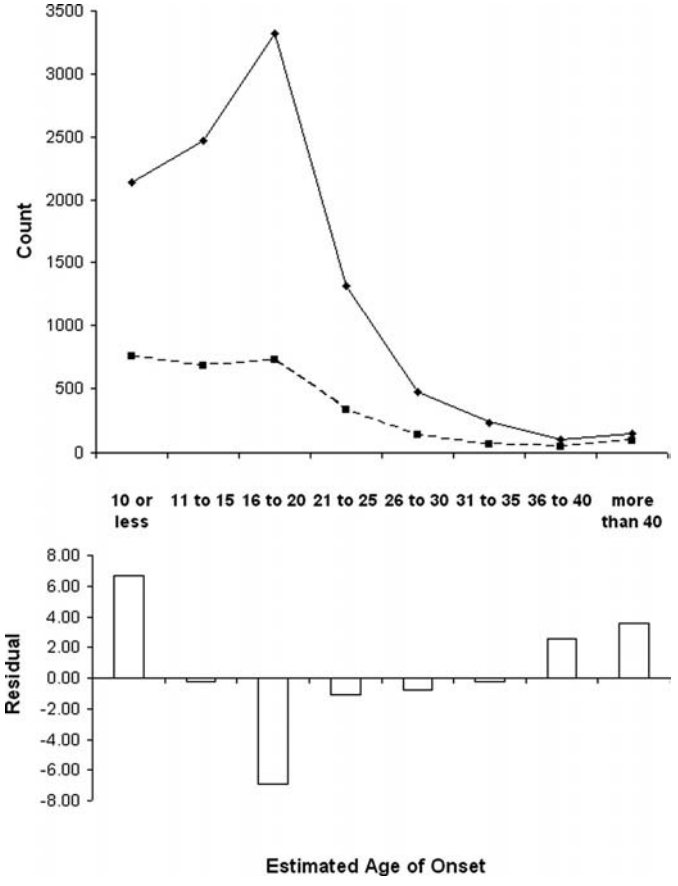


Figure 1 Distribution of estimated age-of-onset of sleep paralysis (SP) episodes for respondents reporting isolated (ISP; *solid line*; N = 10224) and respondents reporting SP with wake-sleep disruption (SPD; *dashed line*; N = 2847).

sleep attacks (Kurtosis = 1.24; S.E. = .186; Z = 3.58; $p < .0002$), and night waking (Kurtosis = 1.69; S.E. = .099; Z = 2.16; $p < .02$). Adjusted standardized residuals generated from a combined chi-square analysis were significantly greater for respondents reporting state-transition disruption for both earlier and later SP onset and less for the peak onset age period of 16–20 than for respondents reporting ISP (Fig. 1).

We had supposed that our respondents, being self-selected, would represent relatively severe cases. Indeed, our sample does contain a higher percentage of severe cases than reported for a recent epidemiological study sample (8), but the similarities are perhaps more striking than the differences (Table 4). In addition, consistent with that earlier study, we found significant, though weak, associations between SP severity and reported narcolepsy diagnosis (particularly with cataplexy), sleep attacks and night waking (Table 4).

Table 4 Percentage of Respondents Reporting Mild, Moderate, and Severe Sleep Paralysis Indexed by Frequency: Chi-Square Tests Compare Isolated Sleep Paralysis (ISP) with SP with Narcolepsy, Sleep Attacks, or Night Waking[a]

	Sleep paralysis severity				
	Mild	Moderate	Severe	χ^2	p
All SP experients					
Present study[b]	60.6	20.3	19.1	—	—
Ohayon et al.[c]	63.5	23.2	13.3	11.03	.004
SP with					
Narcolepsy without cataplexy	0.9	1.6	1.5	7.71	.021
Narcolepsy with cataplexy	0.2	0.3	1.2	14.78	.001
Sleep attacks	4.4	4.8	8.3	56.78	.001
Night waking	17.3	18.3	22.5	33.89	.001

[a]Mild, <1 per month; moderate, >1 per month, but <1 per week; severe, ≥ 1 per week.
[b]Present study N = 13071.
[c]Ohayon et al. (8); N = 469.

One explanation for the association of narcolepsy and SP frequency is that narcolepsy is associated with many more sleep-wake state transitions and hence more opportunities for SP episodes (9). Thus, we asked if multiple state transitions, as indexed by sleep attacks and night waking, were associated with SP frequency over and above the effects of narcolepsy. Linear regression analyses revealed that narcolepsy (without and with cataplexy), sleep attacks and night waking all made independent contributions to SP frequency (Table 5). Both the independent and cumulative effects are, however, very small.

A principle components factor analysis with varimax rotation of this sample confirmed the usual factor structure for both ISP and SP with narcolepsy (without and with cataplexy), night waking and sleep attacks. Indeed, the results were virtually identical to those for the ISP group (Table 1). A repeated-measures ANOVA with narcolepsy as a between groups factor, hallucination type as a within factor, and intensity as dependent variable, indicated that narcolepsy was significantly associated with more intense hallucinations, particularly accelerative V-M hallucinations (Table 6). When the analysis was repeated with age and SP frequency as covariates, the effect of narcolepsy was still significant. When sleep attacks and night waking were used as covariates, however, the effect of narcolepsy was no longer significant. Parallel analyses for sleep attacks and

Table 5 Independent Effects of Narcolepsy (Without and with Cataplexy), Sleep Attacks, and Night Waking on Frequency of SP

	ß	t	p
Narcolepsy without cataplexy	.026	2.94	.003
Narcolepsy with cataplexy	.049	5.45	.001
Sleep attacks	.047	5.14	.001
Night waking	.043	4.83	.001

Overall R = .10; F (4, 13066) = 31.16; $p < .0001$.

Table 6 Estimated Mean Intensity Ratings (Square-Root Transformed, Standard Errors in Parentheses) for Intruder, Incubus, and Vestibular Motor (V-M) Hallucinations for Sleep Paralysis Experients not Reporting or Reporting Narcolepsy with and Without Cataplexy and Significance Tests from ANOVAs[a]

	Narcolepsy with cataplexy	Narcolepsy without cataplexy	No narcolepsy	F[b]	*p*
Intruder	2.05 (.49)	1.93 (.54)	1.86 (.52)	4.47	.011
Incubus	1.79 (.57)	1.89 (.50)	1.76 (.50)	5.66	.003
Accelerative	1.67 (.59)	1.53 (.56)	1.42 (.51)	10.18	.001
V-M	1.82 (.64)	1.65 (.58)	1.61 (.55)	4.46	.012
Interaction:		$F(3, 39195) = 2.21$, $p < .04$			

[a]See text.
[b]df = 1, 13065.

night waking yielded significant effects for intensity as well as a significant interaction for hallucination type and sleep attacks but not for night waking or narcolepsy. Sleep attacks were especially associated with V-M hallucinations (Table 7).

The association of night-waking with intensity of V-M hallucinations may simply reflect that SP episodes occur at night and in the dark, causing the experient to focus more on the accompanying sensations. The effects of sleep attacks (and narcolepsy with cataplexy) on intensity, however, were mainly for V-M hallucinations, possibly reflecting greater vestibular activation following loss of muscular control and support in awkward positions during naps or cataplectic attacks relative to nighttime sleeping. To further test for the effects of context, an ANOVA was conducted for respondents reporting SP episodes exclusively during naps and for those reporting episodes only during the main period of sleep with type of hallucinations as repeated measures and intensity as the dependent variable. Age, SP frequency, narcolepsy, night waking, and sleep attacks were all used as covariates. There was a significant interaction of intensity for time of episode and type of hallucination. Consistent with expectations based on the effects of night waking and sleep attacks, episodes during the main period of sleep were particularly associated with more intense Intruder hallucinations, whereas naps were associated with more intense V-M experiences (Table 8). The differences in all cases are, however, rather small.

Table 7 Estimated Mean Intensity Ratings (Square-Root Transformed, Standard Errors in Parentheses) for Intruder, Incubus, and Vestibular Motor (V-M) Hallucinations for Sleep Paralysis Experients not Reporting or Reporting Sleep Attacks and Significance Tests from ANCOVA[a]

	Sleep attacks	No sleep attacks	F[b]	*p*
Intruder	1.88 (.005)	1.86 (.021)	0.40	.525
Incubus	1.80 (.004)	1.76 (.020)	4.53	.033
Accelerative	1.51 (.005)	1.42 (.005)	18.17	.001
V-M	1.70 (.022)	1.60 (.005)	19.32	.001
Interaction:	$F(3, 39195) = 5.43$, $p < .001$			

[a]See text.
[b]df = 1, 13065.

Table 8 Estimated Mean Intensity Ratings (Square-Root Transformed, Standard Errors in Parentheses) for Intruder, Incubus, and Vestibular Motor (V-M) Hallucinations for Sleep Paralysis Experients Reporting SP Episodes Exclusively During the Main Period of Sleep or During Naps and Significance Tests from ANCOVA (Controlling for age, SP Frequency, Narcolepsy, Night Waking, and Sleep Attacks.)

	Main	Nap	F[a]	*p*
Intruder	1.86 (.005)	1.73 (.021)	78.99	.0001
Incubus	1.74 (.004)	1.67 (.020)	23.74	.0001
Accelerative	1.39 (.005)	1.34 (.005)	15.25	.0001
V-M	1.54 (.022)	1.61 (.005)	21.38	.0001
N	6792	1604		
Interaction:	$F(3, 25167) = 50.85$, $p < .0001$)			

[a]df = 1, 8389.

III. Conclusions

There are several implications to be drawn from these results. First, measures of sleep-wake state transition disruption are associated with the severity of SP, both in terms of frequency of SP episodes and qualitative differences in the intensity of hallucinations. These effects appear to be consistent with the view that frequency of SP is affected, in part, by dysregulation of sleep-wake state transitions such as might be associated with narcolepsy (10). This suggests that the association between narcolepsy and SP might be indirect and incidental, reinforcing doubts about the appropriateness of including SP and its associated hallucinations as part of the narcoleptic tetrad/pentad. Many previous surveys have indicated that SP is much more common than narcolepsy and that estimates of SP prevalence in the general population often approach or equal those reported for narcoleptic samples (3). Consistent with these earlier studies, cases of narcolepsy constitute a very small minority of SP cases, in the present sample, and the differences between the SP experiences of experients with and without narcolepsy are few and small. Moreover, most of these effects can be explained by frequent sleep-wake state transitions among narcoleptic patients, which would simply provide many more opportunities for episodes in susceptible individuals. The relation between sleep-wake state transition disruption and chronicity as well as early and late onset of SP is also consistent with this hypothesis. Moreover, different types of disruption also appear to lead to different contexts in which episodes can occur and hence, indirectly, affect the intensity and quality of hallucinoid experiences associated with SP (9). Further studies of both the timing within the sleep cycle and circadian patterning of SP episodes among both clinical samples and among the general population are clearly suggested by these findings.

References

1. Thorpy MJ. ed. International Classification of Sleep Disorders: Diagnostic Coding Manual. Rochester MN: American Sleep Disorders Association, 1990.
2. Hishikawa Y, Shimizu T. Physiology of REM sleep, cataplexy, and sleep paralysis. Adv Neurophysiol 1995; 67:245–269.

3. Cheyne JA, Rueffer SD, Newby-Clark IR. Hypnagogic and hypnopompic hallucinations during sleep paralysis: Neurological and cultural construction of the night-mare. Conscious Cogn 1999b; 8:319–337.
4. Cheyne JA. Sleep paralysis and the structure of waking-nightmare hallucinations. Dreaming 2003; 13:163–179.
5. Cheyne JA, Girard TA. Spatial Characteristics of Hallucinations Associated with Sleep Paralysis. Cogn Neuropsychiatry 2004; 9:281–300.
6. Whalen PJ. Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. Curr Dir Psychol Sci 1998; 7:177–188.
7. Maquet P, Péters JM, Aerts J, Delfiore G, Degueldre C, Luxen A, Franck G. Functional neuroanatomy of human rapid-eye-movement sleep and dreaming. Nature 1996; 383:163–166.
8. Ohayon MM, Zulley J, Guilleminault C, Smirne S. Prevalence and pathological associations of sleep paralysis in the general population. Neurology 1999; 52:1194–1200.
9. Cheyne JA. Situational Factors Affecting Sleep Paralysis and Associated Hallucinations: Position and Timing Effects. J Sleep Res 2002; 11:169–177.
10. Nishino S, Riehl J, Hong J, Kwan M, Reid M, Mignot E. Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. Neurosci Res 2000; 38:437–446.

13
Epidemiology of Narcolepsy: Development of the Ullanlinna Narcolepsy Scale

CHRISTER HUBLIN
Finnish Institute of Occupational Health, Brain@Work Research Center, Helsinki, Finland

I. Prologue

Until the beginning of the 1990s there were only about one dozen studies on the prevalence of narcolepsy, the earliest published in 1945 by Solomon (1). Despite great differences in definitions of the disorder, study populations, diagnostic procedure, and other methodological aspects, the results gave quite a remarkable 2500-fold difference in the prevalence, ranging from 0.23 to 590 narcoleptic subjects per 100,000 of population. Nevertheless, 95% confidence intervals could be calculated in eight studies and they overlapped or nearly overlapped in seven of them, indicating that the prevalence probably is in the range of 10–100 per 100,000.

Generally, questionnaire-based prevalence studies had given higher figures than those with clinically and polygraphically confirmed diagnosis, most probably caused by lower diagnostic accuracy. Because of relative low frequency of narcolepsy, a prevalence study must be performed in a large population sample, which may lead to a need for a large number of expensive and time-consuming sleep laboratory studies. Therefore, a reliable selection method for large epidemiological studies would be necessary, and our aim was to try to develop such a screening tool.

II. The Finnish Twin Cohort and the Research Group

There is a long tradition of epidemiological research in Finland. One of the most productive groups has worked in the Finnish Twin Cohort, led from its start in the mid 1970s by two specialists in epidemiology, Profs. Jaakko Kaprio and Markku Koskenvuo (2). Sleep has been one of the research topics since the beginning of 1980s when Dr. Markku Partinen joined the Cohort. This author was incorporated to the group in the end of 1980s, when preparing a thesis on narcolepsy under supervision of Dr. Partinen. In addition to a prevalence study, the other main goal was to find monozygotic twin pairs for further analysis, planned in cooperation with the Stanford Sleep Research Center, U.S.A.

The third large questionnaire administered to the Older Finnish Twin Cohort in 1990 included 22 sleep- and vigilance-related questions, for example, on napping,

falling asleep in different situations, sleep latency in the evening, and manifestations of sudden muscle weakness associated with strong emotions. These questions covered the two main symptoms of narcolepsy, abnormal sleep tendency and cataplexy, and we planned to use some set of these responses to develop a screening method to identify subjects fulfilling the minimal diagnostic criteria of narcolepsy according to the ICSD.

III. Where Does the Name Ullanlinna Come From?

Originally, Ullanlinna is the name of a Southern district of the city Helsinki, capital of Finland. A sleep laboratory organized by Dr. Partinen was situated at Ullanlinna Service Center which was owned by Miina Sillanpää Foundation. Thus, the origin of the name is Ullanlinna Sleep Clinic and Research Center, where we did most of our sleep recordings between the mid-1980s and mid-1990s. The Ullanlinna Sleep Laboratory is part of the history of Finnish sleep research; it is well justified that its name continues to live in the name of the narcolepsy screening tool.

IV. The Development of the Ullanlinna Narcolepsy Scale (UNS)

Based on clinical experience, some combinations of questions from the Twin Cohort questionnaire were assessed, and finally four questions with eleven items were included in the UNS. First the UNS (range of the scale 0–44) was tested in a sample of patients with narcolepsy-cataplexy (N = 24; mean, 27.30; range, 14–41) and in the study population (N = 11.354; mean, 5.37; range, 0–39). Later during the validation procedure the UNS was tested in several patient and other comparison groups (sleep apnea, multiple sclerosis, depression, epilepsy, sciatica, alcohol abuse, subjects with neurovegetative symptoms, EDS, and insomnia, total N = 2459), with mean values mostly between 4 and 6. In many groups there were a slight or near overlap compared to the narcolepsy range, and more overlap with the sleep apnea group (mean, 9.63; range, 2–28).

The results provided evidence that the UNS, assessing the two main features of narcolepsy, the abnormal sleep tendency and cataplexy, reliably distinguishes patients with narcolepsy from several other groups, with symptoms critical in differential diagnosis of narcolepsy. When using the lowest UNS score among patients with narcolepsy (14) as the cut-point, the method showed high specifity (98.8%) and sensitivity (100%) in the analyzed subjects (3).

V. The Finnish Prevalence Study

The Older Finnish Twin Cohort, representable of the Finnish general population, was used as the population sample (Fig. 1). The UNS score could be computed for 11,354 subjects (mean age 44 years, 54.4% women, 90.8% of all respondents). There were 75 subjects (0.66% of the study population) with an UNS score as high or a higher score ($\geq$14) as the lowest in the validation group with narcoleptics. Those

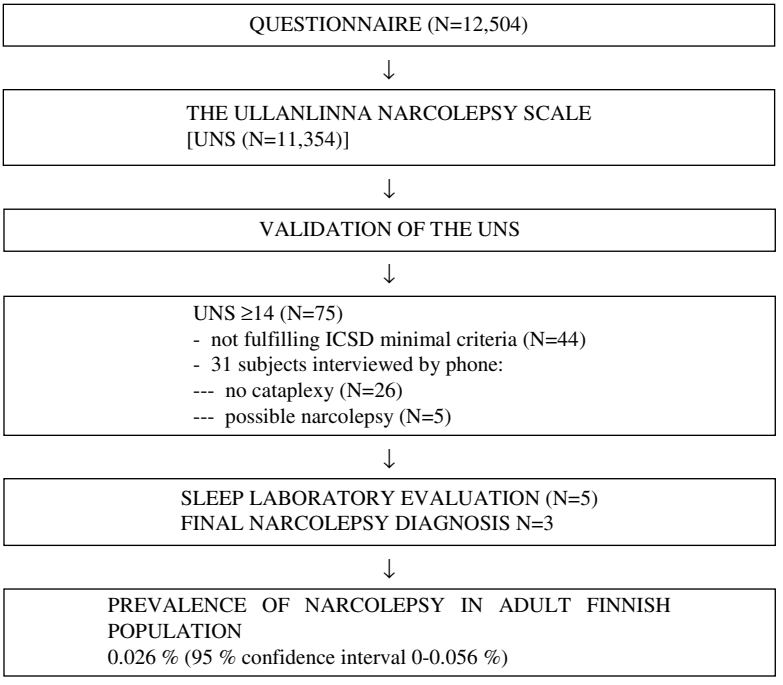

QUESTIONNAIRE (N=12,504)
THE ULLANLINNA NARCOLEPSY SCALE
[UNS (N=11,354)]
VALIDATION OF THE UNS
UNS ≥14 (N=75)
- not fulfilling ICSD minimal criteria (N=44)
- 31 subjects interviewed by phone:
--- no cataplexy (N=26)
--- possible narcolepsy (N=5)
SLEEP LABORATORY EVALUATION (N=5)
FINAL NARCOLEPSY DIAGNOSIS N=3
PREVALENCE OF NARCOLEPSY IN ADULT FINNISH POPULATION
0.026 % (95 % confidence interval 0-0.056 %)

Figure 1 The stepwise progression and decrease in number of subjects investigated in the different phases of the prevalence study.

with any of the following features (revealed by the questionnaire) not compatible with the narcolepsy diagnosis were excluded from further evaluation:

- no cataplexy-like symptoms; or
- unable to nap or difficulties to initiate sleep almost every evening; or
- daytime sleep episodes less than every other day.

The remaining subjects (N = 31) with a high UNS score were contacted by phone for a semi-structured interview. Regarding cataplexy the subjects were first asked about the abruptness of the muscle weakness, and its association with other possible disorders, symptoms or emotions (especially laughter). Only after these questions (to avoid information contamination) a typical cataplectic attack was described, and finally it was asked if the subject ever had had such an attack, with a negative response from 26 subjects. The remaining five subjects considered possible narcoleptics after the telephone interview were called to sleep laboratory evaluation.

The data of the three subjects with the final narcolepsy diagnosis are given in the Table 1. In addition, their nocturnal sleep recordings were unremarkable. All three were dizygotic, discordant for narcolepsy and non-familial cases based on their history. The UNS scores of their co-twins were 4, 8 and unknown (due to refusal to answer the twin health questionnaire). In remaining two subjects out of five (35- and 50-year-old females with UNS scores 23 and 14) the reported daytime sleepiness was not verified

Table 1 Subjects with Confirmed Narcolepsy Diagnosis

Age	Sex	UNS	AS	CPL	HH	SP	S1-lat	REM	DR2/ DQB-0602	Other diseases
52	F	39	32	35	35	46	4.0	1/4	+	Lung adenocarcinoma (at 46 yrs); died of myocardial infarction in 1992 (at 52 yrs)
55	M	20	23	45	30?	—	2.5	3/4	+	Infarctus cordis (at 44 yrs)
43	F	28	15	15	15	15	3.1	2/4	+	In situ cervix (at 35 yrs) and thyroid carcinoma (at 39 yrs)

Abbreviations: F, female; M, male; UNS, Ullanlinna Narcolepsy Scale score value; Age at the symptom onset (AS, abnormal sleep tendency; CPL, cataplexy; HH, hypnagogic hallucinations; SP, sleep paralysis; —, not present; S1-lat, mean Stage 1 latency in minutes; REM—number of SOREMPs on the MSLT; +, positivity on HLA typing.

by sleep logs kept before the sleep registrations, which also were normal in both. Their detailed histories of the emotion associated muscle weakness suggested primarily physiologic cataplexy-like symptoms. No specific diagnoses were made.

Thus, the prevalence of narcolepsy-cataplexy in adult Finnish population is 26 per 100,000 (95% confidence interval 0–56 per 100,000) (4).

VI. Conclusions

Symptoms resembling those occurring in narcolepsy or even combinations of such symptoms are more common in population than the actual syndrome. This limits the use of questionnaires as the only instrument in prevalence studies of narcolepsy. However, the validated questionnaire, the Ullanlinna Narcolepsy Scale, proved to be useful in screening subjects with possible narcolepsy, as the diagnosis could be excluded in >99% of the study population (Figure). The prevalence of narcolepsy in adult Finnish population is 26 per 100.000, and this figure represents the minimum frequency of narcolepsy-cataplexy with clinically significant symptoms.

VII. Epilogue

Dr. Wing and his co-workers have translated and validated the Chinese version of the UNS (CUNS, 5). They found the best cut-off at 13/14 with a sensitivity of 94.1% and specificity of 93.5%, and in principal component analysis two factors that accounted for 45.5% of the total variance. The internal consistency is satisfactory with Cronbach's alpha of 0.75. They concluded that the CUNS has satisfactory psychometric properties and suggested that the UNS could be used across ethnic groups. Later this group used CUNS in an identical prevalence study as the Finnish one, and the prevalence rate was 34 per 100,000 (95% confidence interval 10–117 per

100,000). They concluded that "the similar prevalence rates of narcolepsy across majority of studies using a stringent epidemiological design indicated that the reported cross ethnic differences in the prevalence rates of narcolepsy are more likely a result of methodological limitations" (6).

Acknowledgments

This work would not have been done without the cooperation of the co-authors of the original articles and the contribution of many other persons. I especially want to mention my closest co-workers Jaakko Kaprio, Markku Koskenvuo, and Markku Partinen, who has also been my tutor in sleep medicine.

References

1. Hublin C, Partinen M, Kaprio J, Koskenvuo M, Guilleminault C. Epidemiology of narcolepsy. Sleep 1994; 17:S7–S12.
2. Kaprio J, Koskenvuo M. Genetic and environmental factors in complex diseases: The Older Finnish Twin Cohort. Twin Research 2002; 5:358–365.
3. Hublin C, Kaprio J, Partinen M, Koskenvuo M, Heikkilä K. The Ullanlinna Narcolepsy Scale: a measure of narcoleptic symptoms. J Sleep Res 1994; 3:52–59.
4. Hublin C, et al. The prevalence of narcolepsy: an epidemiologic study in the Finnish Twin Cohort. Ann Neurol 1994; 35: 709–716.
5. Wing YK, Li RHY, Ho CKW, Fong SYY, Chow LY, Leung T. A validity study of Ullanlinna Narcolepsy Scale in Hong Kong Chinese. J Psychosom Res 2000; 49:355–361.
6. Wing YK, Li RHY, Lam CW, Ho CKW, Fong SYY, Leung T. The prevalence of narcolepsy among Chinese in Hong Kong. Ann Neurol 2002; 51:578–584.

14
Epidemiology of Narcolepsy

MAURICE M. OHAYON
Stanford Sleep Epidemiology Research Center, School of Medicine, Stanford University, Stanford, California, U.S.A.

Narcolepsy, a lifelong neurological disorder, has been known for more than a century (1). Its prevalence has been estimated in different parts of the world. However, the findings have varied partly because different methodologies were used and partly because estimates were done using different populations.

I. Prevalence in the United States

Four studies have been undertaken in the United States (Table 1). The first study (2) was performed among young black naval recruits. This early study reported a prevalence of narcolepsy with cataplexy at 0.02% (2 subjects on 10,000) and 3 on 100,000 among white individuals. Two other studies (3,4) recruited participants through advertisement in newspapers (3) or television broadcasts and then through telephone interviews assessed the presence of narcolepsy. Afterward, the prevalence of narcolepsy was extrapolated to the general population with rates of 0.05% and 0.067%, respectively.

A recent study (5) used the records-linkage system of the Rochester Epidemiology Project to review all medical records entered in that system between 1960 and 1989. All patients were living in Olmsted County. Each medical record was coded into the system using the International Classification of Diseases. Medical records were then classified as "Definite Narcolepsy," "Probable Narcolepsy (laboratory confirmation)" or "Probable Narcolepsy (clinical)." Prevalence of narcolepsy (with or without cataplexy), extrapolated to the 1985 Olmsted County population, was set at 0.056% and prevalence of narcolepsy with cataplexy was set at 0.035%. This is the only study that calculated the incidence of narcolepsy. They found an incidence of 1.37/100,000 per year (1.72 for men, 1.05 for women). Moreover the incidence rate was the highest in the second decade, followed in descending order by the third, fourth and first decades.

II. Prevalence in Europe

Six studies have been conducted in European populations. The oldest study was performed in 1957 by Roth (6). Based on a review of his patient material, he extrapolated that the prevalence of narcolepsy in Czechoslovakia was between 0.02% and 0.03%.

Table 1 Prevalence of Narcolepsy in the United States

Authors	Population	N	Age range	Methods	Prevalence per 100,000
Solomon, 1945 (2)	Black Americans	10,000	16–34	Navy recruit men	20
Dement et al., 1972 (3)	San Francisco area, California	Unknown	Unknown	Population sample, newspaper advertisement, telephone interview	50[a]
Dement et al., 1973 (4)	Los Angeles area, California	Unknown	Unknown	Population sample, TV advertisement, telephone interview	67[a]
Silber et al., 2002 (5)	Olmsted County, Minnesota	Unknown	0–109	Review of patients' charts of the Rochester Epidemiology Project	56[a]

[a]Prevalence was extrapolated.

Table 2 Prevalence of Narcolepsy in Europe

Authors	Population	N	Age range	Methods	Prevalence per 100,000
Roth, 1980 (6)	Czech Caucasians	Unknown	Unknown	Patient material, polysomnography	20[a]
Franceschi et al., 1982 (7)	Italy	2,518	6–92	Unselected in-patients, questionnaire, polysomnography	40
Billiard, 1987 (8)	Vincennes and Tarascon, France	58,162	17–22	Male military recruits, questionnaire	55
Hublin et al., 1994 (9)	Finland	12,504	33–60	Twin cohort, postal questionnaire, telephone interview, polysomnography, HLA typing	26
Ondzé et al., 1998 (10)	Gard department, France	14,195	> 15	Patients of all physicians of Gard department. Questionnaire + follow-up by phone interview and more detailed questionnaire	21
Ohayon et al., 2002 (11)	U.K., Germany, Italy, Portugal and Spain	18,980	15–100	Representative sample of general population. Telephone interview with Sleep-EVAL system	47

[a]Prevalence was extrapolated.

Table 3 Prevalence of Narcolepsy in Asia

Authors	Population	N	Age range	Methods	Prevalence per 100,000
Honda, 1979 (12)	Japan	12,469	12–16	School sample, questionnaire	160
Tashiro et al., 1994 (13)	Japan	4,559	17–59	Sample of employees, questionnaire, personal interview	180
Wing et al.,1994 (14)	China	342	> = 18	Patient material, polysomnography and HLA typing	1 to 40[a]
Han et al., 2001 (15)	China	70,000	5–17	Consecutive patients attending a pediatric neurology clinic. Screening questionnaire + polysomnography, MSLT and HLA typing	40
Wing et al., 2002 (16)	Hong Kong, China	9,851	18–65	Random telephone survey using the Chinese version of the Ullanlinna Narcolepsy Scale + MSLT + HLA typing	34

[a]Prevalence was extrapolated.

This estimate also included "monosymptomatic" patients. When only patients with narcolepsy with cataplexy were included, the prevalence was between 0.013% and 0.02%.

A study of excessive daytime sleepiness reviewed the charts of 2,518 unselected patients, aged 6–92 years, admitted to an Italian general hospital during a one-year period. A review of case histories, and clinical and polysomnographic data, revealed one case of narcolepsy. The authors extrapolated the prevalence of narcolepsy at 0.04% in this population (7).

Another study was performed with 58,162 young men recruited for military service in Vincennes and Tarascon (France) (8). Based on the answers to a questionnaire, narcolepsy, defined as more than two daytime sleep episodes per day accompanied by cataplexy and sleeping difficulties, was found in 0.055% of the sample.

A study examined the prevalence of narcolepsy inside the Finnish Twin Cohort (9); 16,179 twin individuals were contacted and 12,504 returned the questionnaire (77.3% response rate). The postal questionnaire included the Ullanlinna Narcolepsy Scale (UNS). Based on the answers to that questionnaire, 75 participants were further interviewed by telephone and then invited to a clinical evaluation, including polygraphic recording and HLA blood typing. Five were strongly suspected of narcolepsy but only three were confirmed by sleep laboratory. This indicates a prevalence of narcolepsy in the Finnish population of 0.026% (95% confidence interval, 0.0-0.06).

Ondzé et al. (10) distributed 38,527 structured questionnaires to all the physicians (general practitioners, specialists, hospital physicians, company physicians and army medical officers) in the Gard department (South of France). The questionnaires were displayed in the waiting rooms and filled out by patients 15 years of age or older; 14,195 questionnaires of patients living in the Gard department were analyzed. A total of 29 subjects were classified as possible narcoleptics and were further interviewed by telephone. Four of them were identified as probable narcoleptics and were HLA-typed. Three of them were confirmed by polysomnography and HLA typing (*DRB*, *1501* and *DQB 0602*) leading to a prevalence of narcolepsy equal to $3/14{,}195 = 0.021$ % in the Gard "département".

Another study investigated the prevalence of narcolepsy in five European countries (United Kingdom, Germany, Italy, Portugal and Spain). These five countries represent 205 million Europeans aged 15 years and over. The study was conducted using telephone interview with the Sleep-EVAL System to administer the questionnaires. The system contained all the questions necessary to validate the criteria required by the ICSD classification for the diagnosis of narcolepsy. Minimal criteria for narcolepsy were defined as the presence of recurrent daytime naps occurring at least twice daily or lapses into sleep for a minimum of three months and the presence of cataplexy (the sudden bilateral loss of postural muscle tone associated with intense emotion). Based on that definition, the prevalence of narcolepsy was 0.047% (95% confidence interval, 0.016% to 0.078%).

III. Prevalence in Asia

Five studies have been performed in Asia (Japan and China). The two oldest studies were conducted in Japan (12,13) and yielded the highest prevalence of narcolepsy.

The first study, a questionnaire-based survey of 12,469 adolescents from Fujisawa, estimated a prevalence of 0.16% for narcolepsy with cataplexy (12). Another study of 4,559 Japanese employees, aged between 17 and 59 years, used a questionnaire, followed by an interview of subjects suspected of having narcolepsy, and polysomnographic examination if appropriate (13). This study reported a prevalence of 0.18%.

Three studies performed in China estimated the prevalence of narcolepsy between 0.001% and 0.04% in adults (14) and at 0.04% in a sample of 70,000 children and adolescents (15). Subsequently, Wing et al. (16) performed another study using a general population sample of 9,851 adults aged between 18 and 65 years. They administered by telephone a validated Chinese version of the Ullanlinna Narcolepsy Scale. Twenty-eight subjects who had positive scores on the Ullanlinna Narcolepsy Scale were invited to a clinical interview and further testing (MSLT and HLA typing). Three subjects refused supplemental evaluation. Three subjects were found to have narcolepsy. This set the prevalence of narcolepsy at 0.034% (95% CI: 0.021%–0.154%).

IV. Prevalence in Middle East

Two studies were conducted in the Middle East: one in Israel (17) and one in South Arabia (18). The lowest narcolepsy frequency was observed among Israeli Jews. In a study of 1,526 patients (2/3 of the subjects were Jewish and 1/3 Arabs) complaining of excessive daytime sleepiness and who were clinically interviewed and polysomnographically recorded, narcolepsy was diagnosed in only six. This sets the prevalence of narcolepsy at 0.002% in the general Israeli Jewish population, a group known for its low rate of human leukocyte antigen (HLA-DR2) (17). Another study was conducted with 23,227 individuals aged 1 year or older living in South Arabia. Interviewers administered a questionnaire in face-to-face interviews. A neurologist subsequently evaluated all participants with abnormal responses in the questionnaire. Narcolepsy was found in 0.04% of the sample (18).

V. Conclusions

Studies that have investigated the prevalence of narcolepsy have reported a prevalence ranging between 0.02% and 0.067% in North America, Europe, Asia and the Middle East, with the exception of the two Japanese studies, where the prevalences were clearly higher than in the other studies. Whether this is a particularity of the Japanese population or a bias due to the methodology remains to be further investigated. The major weakness of these two studies remains the assessment of cataplexy based on a single question assessing muscle weakness during a strong emotion. Questionnaire-based studies have shown that episodes of muscle weakness triggered by emotions are reported by up to 30% of the general population (8,9,11,12). These episodes are generally not genuine cataplexy. Further investigation is needed to determine which muscles are involved, which emotions triggered the episode, and what were the frequency and last occurrence of episodes. Furthermore, it should be stressed that epidemiological surveys completed with polysomnography yield lower prevalences than studies based only on questionnaires.

The importance of genetic factors in narcolepsy has been addressed for more than 60 years (19). However, the results varied from six to 40 percent of narcoleptic individuals who have a close relative with the disease (20–24). The risk for narcolepsy was estimated to be between 10–40 times higher among families with a narcoleptic member than in the general population (20). However, other factors were also cited as playing a role in the appearance of narcolepsy. This was further illustrated in twin studies (25–27). Among 20 pairs of narcoleptic monozygotic twins, only 25–30% were concordant for narcolepsy-cataplexy (28).

Limitations from existing classifications pose serious difficulties in studying narcolepsy in the general population. The use of too large criteria inflates the prevalence. With the exception of cataplexy, the other narcolepsy symptoms (automatic behavior, sleep paralysis, hypnagogic hallucinations) are too poorly defined to be useful in epidemiology. Furthermore, recurrent intrusions of elements of REM sleep into the transition between sleep and wakefulness are highly prevalent in the general population: 6.2% for sleep paralysis and 24.1% for hypnagogic hallucinations (11). This indicates that these symptoms are not specific to narcolepsy (29,30).

References

1. Gélineau J. De la narcolepsie. Gaz des Hop (Paris) 1880; 53:626–628, 635–637.
2. Solomon P. Narcolepsy in negroes. Dis Nerv Syst 1945; 6:176–183.
3. Dement W, Zarcone W, Varner V et al. The prevalence of narcolepsy. Sleep Res 1972; 1:148.
4. Dement WC, Carskadon M, Ley R. The prevalence of narcolepsy II. Sleep Res 1973; 2:147.
5. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. Sleep 2002; 25:197–202.
6. Roth B, Eng. Trans. Broughton R. Narcolepsy and Hypersomnia. Chapter 10. London 1980.
7. Franceschi M, Zamproni P, Crippa D, Smirne S. Excessive daytime sleepiness: a 1-year study in an unselected inpatient population. Sleep 1982; 5:239–247.
8. Billiard M, Alperovich A, Perot C, Jammes C. Excessive daytime sleepiness in young men: prevalence and contributing factors. Sleep 1987; 10:297–305.
9. Hublin C, Kaprio J, Partinen M, Koskenvuo M, Heikkila K, Koskimies S, Guilleminault C. The prevalence of narcolepsy: an epidemiological study of the Finnish Twin Cohort. Ann Neurol 1994; 35:709–716.
10. Ondzé B, Lubin S, Lavendier B, Kohler F, Mayeux D, Billiard M. Frequency of narcolepsy in the population of a French "département". J Sleep Res1998; 7: 193.
11. Ohayon MM, Priest RG, Zulley J, Smirne S, Paiva T. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. Neurology 2002; 58:1826–1833.
12. Honda Y. Census of narcolepsy, cataplexy and sleep life among teenagers in Fujisawa city. Sleep Res 1979; 8:191.
13. Tashiro T, Kanbayashi T, Iijima S, Hishikawa Y. An epidemiological study on prevalence of narcolepsy in Japanese. J Sleep Res 1992; 1(suppl):228.
14. Wing YK, Chiu HF, Ho CK, Chen CN. Narcolepsy in Hong Kong Chinese–a preliminary experience. Aust N Z J Med 1994; 24:304–306.
15. Han F, Chen E, Wei H, Dong X, He Q, Ding D, Strohl KP. Childhood narcolepsy in North China. Sleep 2001; 24(3):321–324.
16. Wing YK, Li RH, Lam CW, Ho CK, Fong SY, Leung T. The prevalence of narcolepsy among Chinese in Hong Kong. Ann Neurol 2002; 51:578–584.
17. Lavie P, Peled, R. Letter to the Editor: Narcolepsy is a Rare Disease in Israel. Sleep 1987; 10:608–609.
18. al Rajeh S, Bademosi O, Ismail H, Awada A, Dawodu A, al-Freihi H, Assuhaimi S, Borollosi M, al-Shammasi S. A community survey of neurological disorders in Saudi Arabia: the Thugbah study. Neuroepidemiology 1993; 12(3):164–178.
19. Krabbe E, Magnussen G. Familial aspects of narcolepsy. Tran Am Neurol Ass 1942; 17:149–173.

20. Nevsimalova S, Mignot E, Sonka K, Arrigoni JL. Familial aspects of narcolepsy-cataplexy in the Czech Republic. Sleep 1997; 20:1021–1026.
21. Billiard M, Pasquie-Magnetto V, Heckman M, Carlander B, Besset A, Zachariev Z, Eliaou JF, Malafosse A. Family studies in narcolepsy. Sleep 1994; 17(8 Suppl):S54–S59.
22. Hayduk R, Flodman P, Spence MA, Erman MK, Mitler MM. HLA haplotypes, polysomnography, and pedigrees in a case series of patients with narcolepsy. Sleep 1997; 20:850–857.
23. Guilleminault C, Mignot E, Grumet FC. Familial patterns of narcolepsy. Lancet 1989; 2(8676): 1376–1379.
24. Baraitser M, Parkes JD. Genetic study of narcoleptic syndrome. J Med Genet 1978; 15:254–259.
25. Honda M, Honda Y, Uchida S, Miyazaki S, Tokunaga K. Monozygotic twins incompletely concordant for narcolepsy. Biol Psychiatry 2001; 49:943–947.
26. Pollmacher T, Schulz H, Geisler P, Kiss E, Albert ED, Schwarzfischer F. DR2-positive monozygotic twins discordant for narcolepsy. Sleep 1990; 13:336–343.
27. Partinen M, Hublin C, Kaprio J, Koskenvuo M, Guilleminault C. Twin studies in narcolepsy. Sleep 1994; 17(8 Suppl):S13–S16.
28. Mignot E. Genetics of narcolepsy and other sleep disorders. Am J Hum Genet 1997; 60:1289–1302.
29. Ohayon MM, Zulley J, Guilleminault C, Smirne S. Prevalence and pathological associations of sleep paralysis in the general population. Neurology 1999; 52:1194–1200.
30. Ohayon MM, Priest RG, Caulet M, Guilleminault C. Hypnagogic and hypnopompic hallucinations: pathological phenomena? Br J Psychiatry 1996; 169: 459–467.

15
Automatic Behavior, Sleep Paralysis, Hypnagogic Hallucinations, Cataplexy: Narcolepsy Spectrum and Alternate Etiologies

MARIANA SZKLO-COXE, TERRY YOUNG, and LAUREL FINN
University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

Sleep paralysis (SP), hypnagogic/hypnopompic hallucinations (HH), and cataplexy, classic symptoms of narcolepsy, have been considered dissociated manifestations of rapid eye movement (REM) sleep (1,2). Although primarily examined in clinical narcolepsy (3) or other sleep disordered patient populations (4–6), these symptoms have also been reported outside of clinical settings and across various countries. For instance, cataplexy-like episodes have been reported in army draftees, and SP in undergraduates, medical students, and shift workers (7–11). While these phenomena have been described in several non-clinical settings, they have been described only rarely in population-based samples (12–15). Moreover, few epidemiologic studies have focused on individual symptoms (13,14), as compared to narcolepsy prevalence estimations. This chapter thus characterizes distributions and correlates of SP, HH, cataplexy, and automatic behavior (AB)—an auxiliary narcolepsy symptom—in adults from a community-based sample. SP, HH, and AB might constitute narcolepsy without cataplexy. Its recent prevalence, based on patients diagnosed with narcolepsy, was estimated at 0.02% (16). To explore the viability of a narcolepsy spectrum, this chapter examines the prevalence of these phenomena and their relationships to narcolepsy's diagnostic and biological markers—sleepiness, nocturnal sleep disruption (3,17), and HLA DQB1*0602, its genetic marker related to a higher risk of developing narcolepsy (18). Along with the tendency to fall directly into REM sleep from wakefulness—expressed as SP, hypnagogic hallucinations, or cataplexy, the unremitting propensity to fall into sleep is a fundamental disturbance of narcolepsy (1). Daytime sleepiness, a key narcolepsy symptom, has been reported in 100% of narcoleptics (3). Disturbed nocturnal sleep (e.g., awakenings) and increased parasomnias (nightmares included) have also been commonly reported in narcoleptics (3,17,19–21), with parasomnias found to co-occur with HH and SP (21). To date, investigations of HLA alleles' relationships to the individual symptoms of SP and/or HH have been limited to clinically-derived (including narcolepsy multiplex families) or specific (e.g., college student) samples (20,22–24). The influence of HLA susceptibility alleles on cataplexy

and other narcolepsy symptoms was evaluated presently to consider these symptoms as constituting a possible narcolepsy spectrum in a population-based sample.

The sleep-related phenomena of interest might, however, be better explained by alternate etiologies which have symptoms in common with narcolepsy (5). Associations of sleep paralysis (SP), hypnagogic/hypnopompic hallucinations (HH), cataplexy, and automatic behavior (AB) with sleepiness might, for example, indicate conditions manifesting sleepiness, e.g., sleep-disordered breathing (SDB) (25), use of hypnotics, snoring, irregular sleep-wake schedules, or sleep difficulties (7). Notably, hypersomnia syndromes—narcolepsy without cataplexy (NwC), idiopathic hypersomnia (IH), and depressive hypersomnia—have been found to overlap (4,26). SP and HH, frequently reported by narcolepsy with cataplexy (NC) patients (74% and 49%, respectively), were also reported by 37% (SP) and 33% (HH) of hypersomnia without cataplexy patients (5). Moreover, SP (clinically- or questionnaire-assessed) and HH (especially clinically-assessed) proportions were similar in IH and NwC, which comprise hypersomnia without cataplexy (4,5). Equal occurrences of SP and HH in hypersomnia patients with (NwC) and without (IH) frequent sleep onset REM periods (SOREMPs) led Aldrich to suggest that, in patients without classic narcolepsy with cataplexy, SP and HH are likely due to factors beyond REM sleep propensity (5). Risk factors which might reflect other conditions and offer explanations beyond narcolepsy for these phenomena were thus investigated in our community-based sample, namely, sleep disturbances, shift work, use of hypnotics and stimulants, and SDB. Insomnia and nightmares, associated with HH and/or SP in European populations (13,14), were presently examined further, with their study extended to cataplexy-like and AB episodes. Given findings of disturbed sleep and shift work triggering SP or analogous phenomena (*ghost oppression, kanashibari, night shift paralysis*) (8,9,11), the links of sleep debt and shift work to SP and other symptoms were also studied. Sleep paralysis, HH, cataplexy, and AB may thus be symptomatic of underlying etiologies sharing narcolepsy symptoms, like sleep deprivation or SDB, or represent independent entities like isolated cataplexy (27) or isolated SP (ISP), including its familial form (22,28). This chapter therefore considers SP, HH, cataplexy (or cataplexy-like phenomena), and AB both within and beyond a narcolepsy context.

II. Methods and Materials

The sample comprised men and women enrolled in the Wisconsin Sleep Cohort Study (WSCS), a longitudinal study of the natural history of sleep disorders, begun in 1989. The sampling frame was a payroll listing of all employees of five state agencies in south central Wisconsin, ages 30–60 at baseline. A two stage random stratified sampling procedure was used (25). Analyses were based on data from 2926 participants who had completed a self-reported mailed survey conducted in 2000. Additional analyses were limited to the following sub-samples (not mutually exclusive): (*i*) $\sim$1000 (DQB1 sample), (*ii*) 811 [polysomnography (PSG) sample], and (*iii*) 764 [Multiple Sleep Latency Test (MSLT) sample] participants on whom complete data were available from the (*i*) mailed survey and DQB1*0602 genotyping, (*ii*) mailed survey, DQB1*0602 genotyping, $\geq$1 overnight protocol, and (*iii*) mailed survey and MSLT, respectively. Participants undergoing a sleep study provided signed informed consent.

The protocol was approved by the University of Wisconsin Medical School-Madison Institutional Review Board. The mailed survey contained items on SP, HH, cataplexy, AB, snoring, shift work, sleep debt, sleep disturbances, and sleepiness. The daytime MSLT (29) was scored by two sleep technicians. The experimental MSLT, conducted after a usual night's sleep, began at ~9 AM with 4 naps at 2-hour intervals and was terminated once the participant fell asleep or at 20 minutes (min), whereas the clinical MSLT, conducted the day after overnight PSG, began ~2 hours after morning wake-up, consisted of 4–5 naps at 2-hour intervals and ended 15 minutes beyond the first sleep epoch, or at 20 min. if no sleep transpired. The 18-channel nocturnal PSG (Grass Heritage PSG Digital Sleep System with Model 15A54 amplifiers), conducted at a sleep laboratory, included electrooculogram, electroencephalogram, electromyography, breathing, arterial oxygenation, among other measures. A trained technician hand-scored each 30-seconds epoch for sleep stage and breathing according to conventional criteria (30). Breathing and oxygenation scores were used to calculate the apnea-hypopnea index (AHI). Whole blood was drawn from subjects the morning after overnight sleep studies. Buffy coat was extracted after centrifugation and stored at -70°C until assay. DNA samples were typed at Stanford for presence of the HLA DQB1*0602 allele, as previously described (31).

Possible response categories for the outcome variables—SP, HH, cataplexy, and AB—were: never, only a few times ever, rarely (<1/month), sometimes (≥ 1/month but <1/week), and often (≥ 1/week). Presence of the outcome was defined as having ever experienced any item describing that symptom ≥ 1/month (vs. <1/month). Self-reported cataplexy was defined as having episodes of muscle weakness in your legs or buckling of your knees with ≥ 1 of the following emotions: (*i*) laughter and/or (*ii*) anger and/or (*iii*) telling/hearing a joke (32). SP was defined as being unable to move your body and feeling paralyzed either upon awakening in the morning and/or upon awakening during night sleep. HH, also referred to as sleep hallucinations, was defined as hearing/seeing strange and frightening things/people when falling asleep at night and/or upon awakening in the morning and/or when drowsy. The combination variable consisted of: cataplexy-alone, SP-alone, sleep hallucinations (HH)-alone, and any 2 or 3 symptoms versus no symptoms. Automatic behavior was defined as times "when you suddenly felt like you 'went blank' with no memory of that period of time" either when driving and/or working at a desk/sitting quietly. Regarding predictors, presence of each survey item—insomnia (difficulty getting to sleep, waking up repeatedly during the night), "feelings of excessive daytime sleepiness" (EDS), nightmares/disturbed sleep—was defined as ≥ 5/month (vs. ≤ 2–4 times/month). Continuous Epworth Sleepiness Scale (ESS) was the sum of eight items (possible range, 0–24). In categorical analyses, ESS > 10 was considered abnormal daytime sleepiness (33). Other survey variables were age (years); gender (male); body mass index (BMI) (weight in kilograms divided by square of height in meters or kg/m^2); sleep debt (week day minus weekend sleep hours) categorized as 1–2 hours, ≥ 3 hours, missing/other (including retired) versus 0 hour; habitual snoring categorized as " ≥ 3 nights/week," "don't know" versus "≤ 1/week but pattern may be irregular"; and shift-work categorized as rotating schedule or night work (sleeping daytime hours), missing/other (retired) versus day work (sleeping nighttime hours). Objective daytime somnolence was measured by the MSLT, with continuous scores computed as average time to sleep onset (min) from 4 (experimental) or 4 to 5 (clinical)

nap trials. MSLT $\leq$5 min was considered abnormal sleepiness; only 4 participants were at 5 min itself (0.6% of MSLT sample). SDB, defined by AHI (number of episodes of apnea and hypopneas per sleep hour (hr)), was categorized as AHI 5–15 (mild SDB), AHI $>$ 15 (moderate-severe SDB) versus AHI $<$ 5 (no SDB). Genotype HLA DQB1*0602 positivity was defined by presence of this homozygous or heterozygous allele. PSG sleep parameters were: (*i*) REM latency (RL), or time (min) from sleep onset to first REM sleep epoch; (*ii*) percent (%) REM, % time in REM sleep/total sleep time (TST); (*iii*) sleep latency (SL), time (min) from lights off to first occurrence of stage 2 or REM sleep; (*iv*) TST (min), as total sleep epochs (30 seconds each); (*v*) wake after sleep onset (WASO), time (min) awake after first sleep onset; (*vi*) sleep efficiency (SE) (%), TST/time (min) in bed from lights out.

III. Analyses

Missing data were deleted from the analyses, except for missing sleep debt and shift work items. Given missing data deleted from models, the number of participants with DQB1 and PSG data ranged from 1001–1018 and 810–812, respectively. Participants reporting much worse night's sleep than usual were excluded from PSG analyses. Since cataplexy is often induced by positive emotions (1,21,32), models of cataplexy when laughing and/or joking but with and without the anger item were compared. As these were similar, anger was maintained in cataplexy's definition. Two additional items, SP when awakening from a nap and HH when napping, were excluded from SP and HH definitions for most analyses to prevent building in associations between SP and HH, on the one hand, and sleep-related variables (e.g., sleepiness), on the other. Nap items were only included in SP and HH definitions for overall prevalence proportion estimates, not for symptom combinations with sleepiness. As symptoms typically do not present concomitantly (3), experiencing symptoms $\geq$few times ever, rather than more frequently, was the focus for prevalence estimations involving symptom combinations. Data were analyzed using SAS (Procedure GENMOD, SAS Institute Inc, Cary, NC) software for descriptive statistics, contingency tables, and regression models. Pearson's chi-square and ordinary *t*-tests compared proportions and means, respectively. Multiple logistic regression models examined adjusted associations of narcolepsy symptoms with EDS, and of DQB1*0602, sleep debt, insomnia, sleepiness (EDS and ESS $>$ 10), nightmares, and SDB with each symptom. Linear regression models examined symptoms' associations with ESS, continuous MSLT, and PSG parameters. Clinical MSLT trends were similar to those combining clinical and experimental MSLTs, so these were not separated. Principal components analyses, especially Varimax rotated solution conducted on the survey sample using SPSS (SPSS Inc, Chicago, Ill), suggested each symptom be modeled as a distinct outcome and was in keeping with conceptualizing symptoms as potentially distinct entities beyond a narcolepsy clinical context.

Odds ratios (ORs) and their 95 percent confidence intervals (95% CI) were calculated from beta coefficient estimates obtained from logistic regression models. An OR expresses the ratio of odds of a given outcome between categories of each independent variable. When the outcome is relatively rare (e.g., $<$5%), the OR approximates the relative risk, or ratio of the probability of the outcome (e.g., SP) in the category of interest (e.g., nightmares) to that in the reference category (e.g., no

nightmares), as in Table 3. When the OR >1, the odds (probability) of the outcome is greater in a given category than in the reference. Two-tailed p-values of ≤ 0.05 were considered significant and used to reject null hypotheses. Final multivariable models included variables with $p \leq 0.05$ and/or key adjustment and/or confounding (beta estimates changed by $\geq 10\%$) variables. All final models were adjusted for age, sex, and BMI. Models of sleep disturbances were also adjusted for snoring; models of SP, HH, cataplexy, and AB predicting MSLT and PSG parameters also for hypnotics and stimulants; and of MSLT ≤ 8 min predicting symptoms also for hypnotics, stimulants, antidepressants, and snoring. Variables neither independently related to outcomes nor likely confounders or intermediaries were excluded from final models and included shift work and DQB1*0602 (models of SDB predicting symptoms); shift work (models of symptoms predicting subjective sleepiness); hypnotics, stimulants, and anti-cataplectic medications (models of symptoms predicting MSLT).

IV. Results

A. Sample Characteristics

Of the survey sample ($n = 2926$), 52.5% were male; 24.7% had ESS > 10; 15.6% reported EDS; 30.2% reported waking up repeatedly during the night, 14.8% difficulty getting to sleep; and 5.4% nightmares/disturbing dreams; 45.8% reported a 1–2 hours sleep debt, 4.9% a ≥ 3 hours debt, and 3.8% missing/other (retired) debt; 40% reported habitual snoring and 6.3% not knowing (for snoring). Day work was reported by 86.0%, rotating shift/night work by 4.2%, and missing/other (retired) by 9.8%. Mean (sd) age was 53 (7.9) years and range 35–77 years; mean (sd) BMI was 28.7 (6.2), range 15.5–66.9 kg/m^2; mean (sd) ESS score was 7.6 (4.3), range 0–24. Of the DQB1 sample ($n = 1001$), 24.3% were DQB1*0602$^+$. Of the PSG sample ($n = 811$), stimulant use was reported by 0.74%, hypnotic use by 5.8%; 97% were Caucasian; 23.3% had mild SDB, 15.4% moderate-severe SDB, and 61.3% no SDB. Means (sd), ranges for PSG parameters were: WASO (min): 63.5 (38.4), 3.0–234.5; TST (min): 379.5 (58.1), 213.5–548.5; % REM: 18.1 (6.1), 0–37.9; RL (min): 122.0 (70.2), 5.0–422.0; SL (min): 12.8 (13.9), 0–124.0; SE (min): 82.4% (9.5), 47.8–98.2. Mean (sd) MSLT ($n = 764$) was 10.9 min (5.0), range 1–20 min; 13.9% of participants had MSLT ≤ 5 min.

B. Narcolepsy Symptoms: Prevalence Proportions and Relationships to Daytime Sleepiness (Epworth Sleepiness Scale >10)

In the survey sample of 2926 participants (Table 1), symptoms maintained relatively similar corresponding relationships to one another across frequency cut-off thresholds of $\geq$few times ever, <1/month but $>$few times ever, and ≥ 1 month. At each cut-off, AB was most prevalent, followed by HH, SP, and cataplexy-like episodes. Prevalence proportions for symptoms reported $\geq$few times ever (history) were: $\sim$57% for AB, $\sim$28% for HH, $\sim$24% for SP, and 10% for cataplexy. Of the sample, 1.3% (95% CI, 0.89%, 1.7%) reported sleepiness and also histories ($\geq$few times ever) of SP, HH, and cataplexy; 3.9% a history of cataplexy with sleepiness; and 3.3% cataplexy history without SP history or sleepiness. Of 113 sleepy participants reporting cataplexy

Table 1 Prevalence Proportions for Self-Reported Sleep Paralysis, Hypnagogic/Hypnopompic Hallucinations, Cataplexy, and Automatic Behavior at Distinct Frequency Cut-offs in a Community-based Sample ($n = 2926$)

Symptoms[a]	Prevalence Proportions (95 percent confidence intervals)
Hypnagogic/hypnopompic hallucinations $\geq$ A few times ever[b]	28.2% (26.6%, 29.9%)
Sleep paralysis$\geq$A few times ever	24.3% (22.8%, 25.9%)
Cataplexy $\geq$ A few times ever	10.0% (8.9%, 11.1%)
Automatic behavior $\geq$ A few times ever	56.8% (55.0, 58.6%)
Hypnagogic/hypnopompic hallucinations $<$1/month but $>$few times ever, or $\geq$1 month	9.1% (8.1%, 10.2%)
Sleep paralysis $<$1/month but $>$few times ever, or $\geq$1 month	5.1% (4.3%, 5.9%)
Cataplexy $<$1/month but $>$few times ever, or $\geq$1 month	2.4% (1.8%, 2.9%)
Automatic behavior $<$1/month but $>$few times ever, or $\geq$ 1 month	18.2% (16.8%, 19.6%)
Hypnagogic/hypnopompic hallucinations $\geq$1/month[c]	3.4% (2.8%, 4.1%)
Sleep paralysis $\geq$1/month	1.6% (1.1%, 2.0%)
Cataplexy $\geq$1/month	1.0% (0.64%, 1.4%)
Automatic behavior $\geq$1/month	8.2% (7.2%, 9.2%)

[a]Each self-reported narcolepsy symptom is defined as follows: *Sleep paralysis*, as being unable to move your body and feeling paralyzed upon awakening in the morning and/or during night sleep and/or from a nap; *Hypnagogic/hypnopompic hallucinations*, as hearing or seeing strange and frightening things or people with sleep onset PM and/or awakening AM and/or when drowsy and/or when taking a nap; *Cataplexy*, as muscle weakness or buckling with laughter and/or anger and/or joke; *Automatic behavior*, as blank spells with no memory of that time period when driving and/or working at a desk/sitting quietly.

[b]$\geq$A few times ever refers to responses of: only a few times ever, $<$1/month, $\geq$1/month but $<$1/week, or $\geq$1/week.

[c]$\geq$1/month refers to responses of: $\geq$1/month but $<$1/week, or $\geq$1/week.

history ($\sim$4% of the sample), 56% also reported SP, 54% also HH, 83.2% also AB, while, of 610 sleepy participants without cataplexy history, 26.9% also reported SP, 30.5% HH, and 71.5% AB. The prevalence (95% CI) for isolated SP (ISP), or SP without cataplexy or sleepiness (10,24), was 13.1% (11.9%, 14.3%), or over half ($\sim$56%) of those with an SP history. Regarding sleepiness prevalence in those with the DQB1*0602 allele ($n = 1001$) or each symptom $\geq$1/month ($n = 2926$) (Figure 1) notably higher proportions of those with cataplexy or AB reported sleepiness–65.5% and 57.3%, respectively, compared to 24.7% reporting sleepiness in the general population. Of those with HH, SP, and the DQB1*0602 allele, 36.8%, 36.4%, and 32.5%, respectively, reported sleepiness. Proportions of sleepiness in those with HH, cataplexy, and AB were significantly ($p < 0.01$) and, with SP, near-significantly higher ($p = 0.07$) than in those without symptoms ($\sim$22–25%). Proportions of sleepiness were the same in those with and without (30%) DQB1*0602.

C. Diagnostic Symptoms and Markers: Excessive Daytime Sleepiness (Subjective and Objective), Nocturnal Sleep, HLA DQB1*0602

Multivariable associations of symptoms $\geq$1/month with daytime sleepiness are presented in Table 2. Regarding subjective sleepiness, HH-alone and combined (any 2

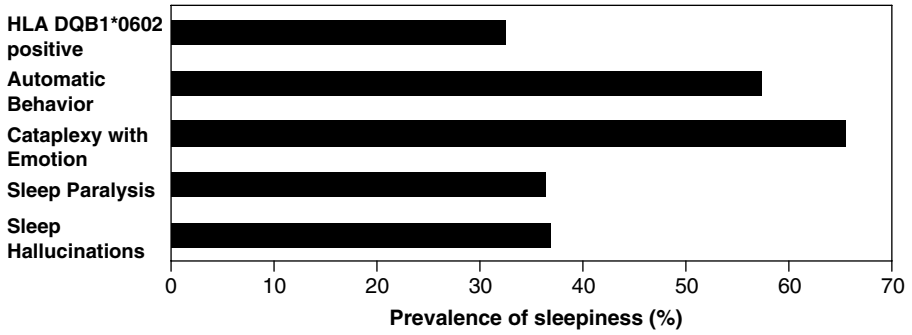


Figure 1 Prevalence (%) of participants with excessive daytime sleepiness (Epworth > 10) for HLA DQB1*0602 and each self-reported narcolepsy symptom ≥1/month.

or 3) symptoms, respectively, were associated with 3-fold and ~9-fold increased ($p \leq 0.0001$) EDS odds, while SP-alone and cataplexy-alone, respectively, with 1.5- and 2.2-fold (non-significantly) increased EDS odds. Compared to no-symptoms (ESS mean, 7.6), cataplexy-alone and combined symptoms were very associated with ESS scores (means, 11–12). The association SP-alone with the MSLT, albeit non-significant (NS), was in the expected negative direction. Contrary to expectation, cataplexy-alone (mean, 13.3 min) and combined (any 2 or 3) symptoms (mean, 16.9 min) were positively related to the MSLT, though only combined symptom findings were significant. MSLT means remained similar upon further adjustments (not shown) for DQB1*0602, hypnotics, stimulants, shift work: HH-alone: 10.4 min, cataplexy-alone: 13.0 min, SP-alone: 8.6 min, combined symptoms: 16.3 min (vs. 10.5 min). As with unadjusted ORs, multivariable ORs (not shown) for MSLT ≤ 8 min (vs. >8 min) as a predictor for HH, SP, and AB ≥1/month (no participants with MSLT ≤ 8 min reported cataplexy ≥1/month) were all non-significant, with ORs for HH and AB <1.0, and only minimally elevated for SP (OR = 1.4). Moreover, PSG findings were unremarkable; no significant differences were observed for any symptom ≥1/month, adjusted for all others, with respect to the PSG outcomes of WASO, RL, percent REM, sleep efficiency, and TST (all p-values > 0.3). Only for HH ≥1/month predicting SL was there a significant difference ($p = 0.05$), with longer SL (16.3 min) for HH versus no-HH (10.4 min). Despite non-significance ($p = 0.25$), AB, like HH, was related to a longer SL, while SP (10.8 min) and cataplexy (12.7 min) were related to slightly shorter SLs versus no-SP (15.9 min) and no-cataplexy (13.9 min). RL was not related to symptoms ≥1/month; percent REM was higher ($p = 0.10$) for cataplexy history with sleepiness (22.3 min, SE = 2.5) versus without (18.1 min, SE = 0.21). Results for the combination variable ≥1/month were nonsignificant (p-values > 0.4). Relative to no-symptoms, sleep efficiency for SP-alone was lower (80%); percent REM (21.0%) and RL (145.3 min) for cataplexy-alone higher; SL for SP-alone shorter (9.7 min), and SL for HH-alone longer (18.7 min)—the latter consistent with any HH (vs. no-HH) results. Proportions of DQB1*0602$^+$ in those with SP, HH, cataplexy, and AB were similar to the 24% in those without these symptoms. In models—both unadjusted and adjusted (for age, sex,

Table 2 Multivariable Associations[a–c] of Self-Reported Narcolepsy Symptoms[d] ≥1/Month with Excessive Daytime Sleepiness (EDS) (Model 1), Epworth Sleepiness Scale (ESS) Scores (Model 2), and Multiple Sleep Latency Test (MSLT) (Model 3) in Population-Based Samples ($n = 2926$ for Models 1 and 2; $n = 764$ for Model 3)

	1. EDS Logistic regression model odds ratio (95% CI)	Mean (score)	**2. ESS** Linear regression model β[e] (95% CI)	Mean (min)	**3. MSLT** Linear regression model β[e] (95% CI)
Narcolepsy Symptoms[d] *vs. none*		*7.6*		*10.9*	
Cataplexy-alone	2.2 (0.67, 7.0)	11.3	3.7 (1.5, 5.8)[b]	13.3	2.3 (−2.5, 7.1)
Sleep paralysisalone	1.5 (0.63, 3.7)	8.2	0.61 (−0.94, 2.2)	9.1	−1.8 (−4.5, 0.92)
Sleep hallucinations-alone	3.1 (3.1, 5.0)[c]	8.6	1.0 (0.05, 2.0)[a]	10.9	−0.03 (−2.2, 2.1)
Any 2 or 3 symptoms	8.8 (3.5, 21.8)[c]	11.7	4.0 (2.2, 5.9)[c]	16.9	6.0 (1.7, 10.3)[b]
Sleep debt[f] *vs. 0 hr*					
1–2 hr	1.3 (1.0, 1.6)[a]		0.45 (0.12, 0.78) [b]		−0.13 (−0.88, 0.62)
≥3 hr	3.3 (2.2, 4.9)[c]		0.93 (0.19, 1.7)[b]		−0.29 (−2.0, 1.4)
Habitual snoring vs. no	1.6 (1.3, 2.0)[c]		1.3 (1.0, 1.7)[c]		0.02 (−0.72, 0.76)

[a] $p \leq 0.05$
[b] $p < 0.01$
[c] $p \leq 0.0001$
[d] *Cataplexy-alone* (prevalence: 0.51% in survey sample, 0.52% in MSLT sample) defined as cataplexy-like episodes without sleep paralysis or hypnagogic/hypnopompic hallucinations, *Sleep paralysisalone* (prevalence: 0.99% in survey sample, 1.7% in MSLT sample) as sleep paralysis without cataplexy-like episodes or hypnagogic/hypnopompic hallucinations. *Sleep Hallucinations-alone* (prevalence: 2.6% in survey sample, 2.8% in MSLT sample) as hypnagogic/hypnopompic hallucinations without sleep paralysis or cataplexy-like episodes, and *Any 2 or 3 symptoms* (prevalence: 0.72% in survey sample; 0.65% in MSLT sample) refers to any cataplexy-like episodes, sleep paralysis, or hypnagogic/hypnopompic hallucinations.
[e] β (regression) coefficient which, in a linear regression model, represents the absolute difference between a given category (e.g., a symptom) and the reference category (e.g., no symptom).
[f] Sleep debt defined as week day minus weekend sleep hours.

BMI), DQB1*0602$^+$ was not significantly related to any symptom $\geq$1/month; adjusted ORs (95% CI) were: 0.95 (0.57, 1.5) for AB, 1.1 (0.38, 3.0) for SP; 1.5 (0.68, 3.3) for HH, 2.1 (0.68, 6.5) for cataplexy. While cataplexy odds were elevated 2-fold in DQB1*0602's presence, they failed to reach significance. Analyses (not shown) of DQB1*0602's associations with these symptoms defined at distinct thresholds, in various combinations, and as unweighted and weighted composite scores (based on principal components analyses) were also non-significant.

D. Risk Factors for Sleep Paralysis, Hypnagogic/Hypnopompic Hallucinations, Cataplexy, and Automatic Behavior $\geq$1/month: Multivariable Analyses

Severe sleep debt ($\geq$3 hour) (Table 3, model 1) was positively related to all symptoms, but the association was only significant for HH ($p = 0.002$). A severe debt increased HH odds by $\sim$200%. The logistic regression beta coefficient for severe debt was decreased by 32% when EDS was added to the model, indicating sleepiness may serve as an intermediary between sleep debt and HH. Those reporting a 1–2 hour debt were 1.8-fold ($p = 0.0006$) and $\sim$2-fold ($p = 0.07$) less likely to have AB and SP, respectively. Snoring was related to $\sim$2-fold increased AB odds ($p = 0.002$), leading to further analyses of ABs link to SDB. Higher BMI was associated ($p = 0.0008$) with AB; for example, a 5 kg/m^2 increase would increase AB odds by $\sim$20%. Younger age was related ($p \leq 0.01$) to increased symptom odds; that is, being 10 years younger would increase HH or SP odds by $\sim$70% and AB odds by85%. Regarding sleep disturbances (Table 3), frequent nightmares/disturbing dreams (model 2) were very positively associated with $\geq$1/month SP, HH, AB ($p < 0.0001$), and with $\geq$1/month cataplexy ($p \leq 0.05$). Insomnia (model 3) was associated positively with all symptoms, with ORs ranging from 1.6 to 3.5 for difficulty falling asleep, and from 1.7 to 2.4 for repeated nocturnal waking. Adding insomnia items to nightmare models (not shown) decreased nightmares' logistic beta coefficient estimates—14% for HH, 23% for SP, 26% for AB, and $\sim$45% for cataplexy, yet nightmares' estimates were still significant ($p \leq 0.003$) for all outcomes but cataplexy ($p = 0.30$). Insomnia may be an intermediary mechanism between nightmares and symptoms, especially cataplexy-like episodes, given the large decrease in nightmare's estimate for cataplexy and its change to non-significance when insomnia items' were added to cataplexy's model. Other insomnia items (not shown) significantly, positively related to narcolepsy symptoms in multivariable models were "very difficult to wake up (am)" and "wake up during the night and have a hard time getting back to sleep," related to SP, HH, AB, and "wake up too early (am) and can't get back to sleep," to HH and AB. Regarding sleepiness (model 4), EDS (ORs, 2.6, 3.9), rather than ESS $>$ 10 (ORs, 1.1, 1.2) was more associated with SP and HH, while ESS $>$ 10 (OR = 4.7), rather than EDS (OR = 2.3), was more so with cataplexy. EDS (OR = 3.4) and ESS $>$ 10 (OR = 3.1) were both strongly associated with AB.

Shift work, hypnotics, stimulants, and sleep-disordered breathing (SDB) were also examined as potential risk factors. Working rotating/night shifts, versus days, was not associated significantly with any symptom $\geq$1/month. Multivariable ORs (95% CI), based on the survey sample, were 0.89 (0.21, 3.75) for SP, 1.2 (0.70, 2.2) for AB, and 1.7 (0.74, 3.7) for HH. Rotating/night shift was associated with 2.8-fold increased odds of cataplexy-like episodes, which did not attain significance but was nearly significant (95% CI, 0.27–21.3). In multivariable models based

Table 3 Multivariable Associations[a] of Sleep Debt and Sleep Disturbances (Nightmares, Insomnia, Daytime Sleepiness) with Self-Reported Narcolepsy Symptoms ≥1/Month in a Population-Based Sample ($n = 2926$): Odds Ratios (OR) and 95% Confidence Intervals (CI) from Multiple Logistic Regression Models

	Sleep paralysis	Hypnagogic/hypnopompic hallucinations	Cataplexy	Automatic behavior
Prevalence	1.5%	3.3%	0.99%	8.2%
Model 1	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sleep debt[b] vs. 0 hr				
1–2 hr	0.53 (0.27, 1.1)	0.97 (0.60, 1.6)	0.62 (0.27, 1.4)	0.56 (0.40, 0.78)[a]
≥3 hr	1.8 (0.71, 4.8)	2.9 (1.5, 5.8)[a]	2.1 (0.75, 5.9)	1.4 (0.87, 2.1)
Habitual snoring vs. no	1.9 (0.98, 3.7)	1.0 (0.64, 1.6)	1.0 (0.55, 2.0)	1.5 (1.2, 1.9)[a]
BMI (5 kg/m^2)	1.1 (0.86, 1.3)	1.1 (0.95, 1.3)	1.1 (0.82, 1.5)	1.2 (1.1, 1.3)[a]
Male vs. female	1.4 (0.74, 2.6)	1.1 (0.69, 1.1)	1.1 (0.49, 2.2)	0.82 (0.62, 1.1)
Age (5 years)	0.77 (0.62, 0.79)[a]	0.77 (0.66, 0.90)[a]	1.1 (0.86, 1.4)	0.73 (0.66, 0.96)[a]
Model 2[c]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Nightmares ≥5 times/month vs. ≤4 times/month	5.4 (2.6, 11.2)[a]	8.8 (5.4, 14.1)[a]	2.9 (1.0, 8.6)[a]	2.5 (1.6, 3.9)[a]
Model 3[c]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Insomnia ≥5 times/month vs. ≤4 times/month				
Difficulty getting to sleep	2.2 (1.2, 4.4)[a]	2.5 (1.6, 4.0)[a]	3.5 (1.6, 7.8)[a]	1.6 (1.1, 2.2)[a]
Repeated nocturnal waking	2.4 (1.3, 4.5)[a]	1.9 (1.2, 3.0)[a]	1.7 (0.77, 3.7)	1.7 (1.3, 2.2)[a]
Model 4[c]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Excessive Daytime Sleepiness				
EDS≥5 times/month vs. ≤4	2.6 (1.3, 5.2)[a]	3.9 (2.4, 6.1)[a]	2.3 (1.0, 5.1)[a]	3.4 (2.5, 4.6)[a]
ESS > 10 vs. ≤10	1.2 (0.60, 2.3)	1.1 (0.71, 1.8)	4.7 (2.1, 13.4)[a]	3.1 (2.3, 4.2)[a]

[a] $p \leq 0.05$

[b] Sleep debt defined as week day minus weekend sleep hours.

[c] Models 2–4 also adjusted for age, sex, BMI, and habitual snoring.

Definitions: *Sleep Paralysis*, as being unable to move your body and feeling paralyzed upon awakening in the morning and/or awakening during night sleep; *Hypnagogic/Hypnopompic Hallucinations*, as hearing or seeing strange and frightening things or people with sleep onset PM and/or awakening AM and/or when drowsy; *Cataplexy*, as muscle weakness or buckling with laughter and/or anger and/or joke; *Automatic Behavior*, as blank spells with no memory of that time period when driving and/or working at a desk/sitting quietly.

Abbreviations: BMI, body mass index; EDS, feelings of excessive daytime sleepiness; ESS, Epworth Sleepiness Scale.

Table 4 Multivariable Associations[a,b] of Sleep-Disordered Breathing with Self-Reported Narcolepsy Symptoms[c] ≥ 1/month in a Population-Based Sample ($n = 810$): Odds Ratios (OR) and 95 Percent Confidence Intervals (95% CI) from Multiple Logistic Regression Models

	Sleep paralysis	Hypnagogic/hallucinations hypnopompic	Cataplexy	Automatic behavior
Prevalence	1.9%	2.8%	0.99%	11.9%
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sleep-disordered breathing[d]				
AHI 5–15 vs. <5	1.0 (0.25, 4.0)	1.1 (0.41, 3.2)	1.4 (0.31, 6.8)	1.1 (0.65, 2.0)
AHI >15 vs. <5	2.1 (0.50, 8.7)	0.85 (0.21, 3.5)	0.49 (0.05, 5.5)	2.0 (1.1, 3.8)[a]
Age (5 years)	0.70 (0.50, 1.1)[c]	0.66 (0.50, 0.90)[a]	1.0 (0.62, 1.6)	0.77 (0.66, 0.90)[a]
Male versus female	1.0 (0.35, 3.0)	1.3 (0.55, 3.2)	1.2 (0.28, 5.5)	0.55 (0.35, 0.88)[a]
BMI (5 kg/m^2)	1.1 (0.70, 1.6)	1.1 (0.73, 1.5)	1.6 (0.95, 2.6)[b]	1.1 (0.95, 1.3)

[a] $p \leq 0.05$.

[b] Near-significant at $p = 0.07$.

[c] Each self-reported narcolepsy symptom is defined as follows: *Sleep Paralysis*, as being unable to move your body and feeling paralyzed upon awakening AM and/or awakening during night sleep; *Hypnagogic/Hypnopompic Hallucinations*, as hearing or seeing strange and frightening things or people with sleep onset PM and/or awakening AM and/or when drowsy; *Cataplexy*, as muscle weakness or buckling with laughter and/or anger and/or joke; *Automatic Behavior*, as blank spells with no memory of that time period when driving and/or working at a desk/sitting quietly.

[d] Mild sleep-disordered breathing (SDB) is defined as AHI 5–15; moderate to severe SDB as AHI > 15.

Abbreviations: AHI, Apnea-Hypopnea Score; BMI, body mass index.

on the PSG sample, stimulants were associated ($p \leq 0.02$) with elevated odds of symptoms: 7.6-fold (95% CI, 1.4, 39.8) for AB, 15.5-fold (95% CI, 1.6, 148.8) for SP, and 21.9-fold (95% CI, 2.1, 228.0) for cataplexy-like episodes. No participants on stimulants reported HH; none on hypnotics reported cataplexy. Hypnotic use was significantly related to SP (OR = 5.4, $p < 0.02$), near-significantly to AB (OR = 1.9, $p = 0.10$), and non-significantly (NS) to HH (OR = 1.7, NS). Compared to no SDB (AHI < 5), mild SDB (AHI 5–15) (Table 4) was not related to any symptoms (ORs, ~1.0–1.4). However, moderate-severe SDB (AHI > 15) was associated with 2-fold increased SP (NS) and AB odds ($p = 0.03$). Adjusting for shift work and DQB1*0602 (not shown) did not alter AHI estimates. BMI was nearly significantly associated with cataplexy ($p = 0.07$).

V. Discussion

Hypotheses that symptoms would be related positively to subjective sleepiness and negatively with the MSLT were only supported for subjective measures. The MSLTs relationship to cataplexy-alone, though non-significant (NS), and to combined symptoms defied expectation, perhaps indicating these are not true narcolepsy symptoms. Though SPs mean MSLT did not meet pathologic sleepiness criteria (<5 min) (29), it neared the narcolepsy cut-off of 8 minutes (6), and its association with the MSLT (albeit NS) was in the expected direction, thus hinting at a link. Nocturnal polysomnographic parameters were largely unrelated to symptoms. As in family members with isolated cataplexy (27), but unlike in narcoleptics (19), REM latencies were higher (yet NS) in those with cataplexy without SP or HH. Neither shorter sleep latencies nor lower sleep efficiencies typical of narcoleptics' sleeps (5,19) were observed. A slightly longer wake after sleep onset (NS) for SP may relate to experimental findings of sleep interruption's role eliciting isolated sleep paralysis (ISP) (24). As HH have been tied to anxiety and dreading bed time (13), anxiety about falling asleep may have contributed to HH reported mainly only when falling asleep at night (~41%) and being related to longer (compared to narcoleptics' shorter) sleep latencies. Symptoms' proportions did not differ by DQB1*0602 status, consistent with similar hypnagogic hallucinations and SP occurrences reported in HLA^+ versus HLA^- patients (23). In contrast to narcoleptics (18), HLA by cataplexy status differences were not found. Overall absence of (and a few unexpected) associations of symptoms with the MSLT, nocturnal sleep, and HLA DQB1*0602^+ suggest symptoms constitute independent entities, like ISP, or express conditions beyond narcolepsy.

Sleep disturbances, hypnotic use, sedative use, and sleep-disordered breathing appear to offer cogent explanations for occurrences of these phenomena in the population presently studied. Severe sleep debt was significantly related to HH, consistent with experimental findings of waking dreams occurring with sleep loss (34). Sleep debt's relationships to increased HH, and possibly (NS) to SP in our community-based sample are consistent with findings from non-clinical samples—sleep disturbance precipitating ISP (HH included in SP episodes) (8,9,35) and night shift paralysis proposed as a "critical incident" for comparing sleep deprivation levels related to distinct shifts (11). Frequent nightmares/disturbing dreams were very predictive of all symptoms. The HH-nightmare tie was consistent with nightmare-sleep

hallucination associations reported in the U.K. population (13), though the stronger link in our sample may be due to the higher nightmare frequency we investigated. Presently, odds were elevated by ~9-fold for HH, suggestive of nightmares as an alternate etiology for HH. However, findings might instead or additionally insinuate a narcolepsy spectrum, given a higher occurrence of nightmare dream content reported in narcoleptics (vs. hypersomnolents or normals) (21) and the high risk of parasomnia development reported in narcoleptics' first-degree family members (nightmares included) (20). Beyond narcolepsy's context, M. Alfred Maury described insomnia as a precipitant for his own hypnagogic visions (36). Presently, insomnia was very positively associated with each symptom. HH results were consistent with U.K. findings—higher proportions of sleep hallucinations in those with insomnia, including self-reported increased sleep latency and disrupted nocturnal sleep (13). This subjective sleep latency finding is consistent with higher PSG-measured SL observed in our WSCS participants with HH (vs. without). The present results for insomnia's relationship to SP are also largely compatible with German and Italian participants with SP more often reporting difficulty falling asleep and disrupted sleep (though insomnia was non-significant in multivariable analyses in this study), and non-restorative sleep's relationship to SP (14). Present findings may also relate to findings of sleep interruption eliciting ISP (24) since, of WSCS participants with SP $\geq$ 1/month during night waking, napping, or morning awakening, most (67%) had SP only during night waking. Also, insomnia may be an intermediary mechanism between nightmares and symptoms (chiefly cataplexy), but cross-sectional data preclude establishing a causal pathway. Insomnia and nightmare effects each existed alone so other pathways deserve exploration. Given the suggested role of disturbed sleep/wake cycle as an SP trigger (8,9,35) and serial night work's relationship to *night shift paralysis* (11), shift work's possible (non-significant) link to cataplexy merits more inquiry. Also, SP and cataplexy are both typified by immobility due to muscle atonia and can be hard to differentiate, especially transitions from one to the other (2).

Both subjective sleepiness measures were strongly associated with AB, consistent with findings of AB as a manifestation of sleepiness and its presence not only in narcoleptic (N), but also in hypersomnolent (H) patients (4,5,21). The 57% of WSCS participants reporting AB resembled the estimate in IH patients (61%) but was higher than estimates in normals (No) (25% to 38%) (4). Positive associations of subjective sleepiness with cataplexy are consistent with findings in H patients (21) and non-clinical groups (7,8). Cataplexy was more related to the Epworth Sleepiness Scale, which measures situational sleep propensities in daily life (33). This may be compatible with a narcolepsy spectrum perspective or instead suggest sleepy non-narcoleptics, as cataplexy-like episodes have been reported more in H than No (21). As active sleep propensity is higher in N than H or No and correlated with cataplexy and AB in N (21), examining this dimension in population-based samples may also help illuminate whether specific symptoms constitute narcolepsy.

Stimulant use was strongly related to increased odds of cataplexy-like episodes, SP, and AB; and hypnotics to increased SP odds, with the latter finding dissimilar to the absence of differences for combined antidepressants/hypnotics previously reported (14). Hypnotics' association (non-significant) to sleep hallucinations resembled that reported in a U.K. study (near-significant) examining combined hypnotics or antidepressants/anxiolytics (13). The ~100% increase in the odds of automatic behavior in the

presence of sleep-disordered breathing (SDB), AB's non-significant associations with narcolepsy's criteria, and its estimate's lack of change with DQB1*0602 adjustment, suggest AB may reflect moderate-severe SDB. BMI being an SDB risk factor may explain BMIs link with AB (25). SDB was non-significantly associated with decreased cataplexy and HH odds, consistent with SDB patients being less likely than other sleepy patients to report HH (5). While males and females had equal odds of reporting symptoms, younger participants were more likely to experience SP, HH, and AB.

Regarding limitations, symptoms were self-reported. In another study, SP, hypnagogic hallucinations, and AB were lower in NwC when questionnaire- versus clinically-assessed (4). Also, symptoms might not be recalled if episodes were infrequent, oscillated, or vanished, fluctuations which occur in narcolepsy's natural history (3). Though symptoms' longitudinal history could not be ascertained from our data, narcolepsy in its developing phase is likely not an issue for WSCSs middle-aged population, as narcolepsy typically originates in life's second decade (3,16). Neither causal nor temporal relationships could be studied due to our data's cross-sectional nature; only indirect inferences could be drawn regarding pathways (e.g., sleep debt $\rightarrow$ sleepiness $\rightarrow$ HH). Insomnia survey items were related to the symptoms examined. Therefore, symptoms' non-associations with nocturnal PSG measures might be due to the smaller PSG sample or to sleep studies being conducted, on average, 9.3 ± 6.9 months (range, 0–728 days) from (before or after) the survey measuring symptoms. A "trick" weakness item was included in the questionnaire to determine whether those responding affirmatively to this item were the ones also reporting cataplexy with anger, laughter, or joking. Cross-tabulation analyses found that the "trick" weakness survey item (muscle weakness in hands) did not overlap with cataplexy items. Also, "trick" weakness' associations with cataplexy were negative (OR, 0.34, $p = 0.10$). As data on hypnagogic SP when falling asleep at night were not collected and ISP is more often hypnopompic (35) than hypnogogic, we may have mainly captured the isolated form of SP, suggested to have a distinct pathophysiology (35) and expression from narcoleptic SP [e.g., ISP is weaker, easier to willingly end (24)]. Still, sleep interruptions can produce SOREMPs and ISP in normals (24); tonic immobility and paradoxical sleep occur in familial and narcoleptic SP (28); and isolated and narcoleptic SP occur with short REM latencies—the latter suggesting a common physiology (24). SP's physiology deserves more elucidation in population-based samples. Sleep characteristics distinguishing narcoleptic subgroups from one another and narcoleptics from hypersomnolents and normals [e.g., dream contents and parasomnias (talking, shouting), tickling as cataplexy trigger] (21) also merit epidemiologic study to further clarify narcolepsy's clinical spectrum in the population.

VI. Conclusions

In the present sample, as in a U.K. sample (13), prevalence proportion estimations for individual symptoms of narcolepsy as well as their combinations were substantially higher than prevalence estimations for narcolepsy (12,15,16). Notably, Wisconsin Sleep Cohort Study estimates for SP (24%) and HH (28%), while lower than in PSG-confirmed classic narcolepsy (3,4), closely resembled self-reported SP (27%) and clinically-assessed hypnagogic hallucinations (30%) in NwC (4), considered a sizeable subgroup of narcolepsy. NwC has been found to comprise 36% of the prevalence

of narcolepsy with and without cataplexy (16). Our HH estimates also resembled HH (28%) reported by hypersomnolent patients (21). In our sample, AB frequencies were similar in excessively sleepy participants with (83.2%) and without (71.5%) cataplexy. Although AB estimates were more prevalent in our community-based participants than in patients studied by Bassetti and Aldrich, our findings resemble theirs with respect to AB being self-reported equally in narcolepsy with (31%) and without (25%) cataplexy (4). WSCS estimates are consistent with those from non-clinical samples, especially for cataplexy history (7), cataplexy without SP or sleepiness (8), cataplexy history with sleepiness (12), and ISP (10). Symptoms' strong relationships to subjective sleepiness, which might be further clarified by examining dimensions like active sleep propensity (SPAS) (21), may indicate they constitute a narcolepsy spectrum. However, in light of symptoms' absence of significant associations with narcolepsy's key markers, subjective sleepiness-symptom links may imply symptoms form independent conditions, like familial SP with sleepiness (22), and/or are manifestations of other etiologies or triggers. Differential relationships of cataplexy-like episodes to the Epworth Sleepiness Scale; and of SP and HH to feelings of excessive daytime sleepiness (EDS) suggest distinct etiologies producing diverse sleepiness experiences, which may relate to recent findings in clinical settings: severity of excessive daytime sleepiness (SPAS score) and cataplexy features permitted identification of narcoleptic subgroups and differentiation from non-narcoleptic EDS patients (including those with cataplexy-like episodes) (21). Overall, present findings suggest symptoms (like cataplexy) typically viewed as pathognomonic for or constituting narcolepsy do not primarily represent narcolepsy expression or susceptibility in the population, but, rather, several other conditions. Clinically-based results indicate hypnagogic hallucinations (clinically-assessed) and SP (questionnaire-or clinically-assessed) do not discriminate IH from NwC (HH, SP) or from NC (SP) (4). Similarly, our community-based findings suggest SP, HH, AB, and cataplexy-like episodes with emotion are not narcolepsy-specific. Sleepiness and insomnia were related to all symptoms, but distinct sleepiness measures were related differentially to symptoms, while certain insomnia items were especially related to HH and cataplexy. The associations of distinct correlates with phenomena—hypnotic use with sleep paralysis, sleep debt with hypnagogic/hypnopompic hallucinations, and sleep-disordered breathing with automatic behavior—imply discrete etiologies and pathophysiologies underlie individual symptoms. Findings pertain to recent work proposing narcoleptic symptom clusters having discrete pathophysiologies (21). Future work will focus on symptoms, susceptibility alleles, and sleep onset REM periods to determine whether specific diagnostic groupings form a narcolepsy spectrum in the population or reflect additional alternate etiologies beyond those studied herein, and whether the latter could be complementary with a spectrum perspective. Contributions of psychiatric disturbances, especially depression, to these symptoms are currently being investigated in our sample.

VII. Funding

This research was supported by National Institutes of Health grants NS23724 and HL62252, and also, in part, through the use of the Prizker Network Research Funds.

Acknowledgments

We are indebted to Kathryn Pluff, Mary Sundstrom, and Katherine Kenison for overnight polysomnography and multiple sleep latency test recordings and scoring, and Amanda Rasmuson and Diane Demonaco Dowd for setting up overnight sleep studies. We also gratefully acknowledge Dr. Javier Nieto for his thoughtful comments regarding this manuscript, Dr. Ling Lin for conducting HLA typing, and Diane Austin, Linda Evans, and Robin Stubbs for their technical expertise and many helpful contributions.

References

1. Hishikawa Y, Kaneko Z. Electroencephalographic study on narcolepsy. Electroencephalogr Clin Neurophysiol 1965; 18,249–18,259.
2. Hishikawa Y, Shimizu T. Physiology of REM sleep, cataplexy, and sleep paralysis. Adv Neurol 1995; 67:245–271.
3. Billiard M, Besset Al, Cadilhac J. The clinical and polygraphic development of narcolepsy. In: Guilleminault C, Lugaresi J, eds. Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution. New York: Raven Press, 1983:171–185.
4. Bassetti C, Aldrich MS. Idiopathic hypersomnia: a series of 42 patients. Brain 1997; 120(part 8): 1423–1435.
5. Aldrich MS. The clinical spectrum of narcolepsy and idiopathic hypersomnia. Neurology 1996; 46(2), 393–401.
6. Moscovitch A, Partinen M, Guilleminault C. The positive diagnosis of narcolepsy and narcolepsy's borderland. Neurology 1993; 43(1):55–60.
7. Billiard M, Alperovitch A, Perot C, et al. Excessive daytime somnolence in young men: prevalence and contributing factors. Sleep 1987; 10(4):297–305.
8. Wing YK, Lee ST, Chen CN. Sleep paralysis in Chinese: ghost oppression phenomenon in Hong Kong. Sleep 1994; 17(7):609–613.
9. Fukuda K, Miyasita A, Inugami M, et al. High prevalence of isolated sleep paralysis: kanashibari phenomenon in Japan. Sleep 1987; 10(3):279–286.
10. Everett HC. Sleep paralysis in medical students. J Nerv Ment Dis 1963; 136:283–287.
11. Folkard S, Condon R. Night shift paralysis in air traffic control officers. Ergonomics 1987; 30(9):1353–1363.
12. Hublin C, Kaprio J, Partinen M, et al. The prevalence of narcolepsy: an epidemiological study of the Finnish Twin Cohort. Ann Neurol 1994; 35(6):709–716.
13. Ohayon MM, Priest RG, Caulet M, et al. Hypnagogic and hypnopompic hallucinations: pathological phenomena? Br J Psychiatry 1996; 169(4):459–467.
14. Ohayon MM, Zulley J, Guilleminault C, et al. Prevalence and pathologic associations of sleep paralysis in the general population. Neurology 1999; 52(6):1194–1200.
15. Ohayon MM, Priest RG, Zulley J, et al. Prevalence of narcolepsy symptomatology and diagnosis in the European general population. Neurology 2002; 58(12):1826–1833.
16. Silber M, Krahn LE, Olson EJ, et al. The epidemiology of narcolepsy in Olmsted Country, Minnesota: a population-based study. Sleep 2002; 25(2):197–202.
17. Mitchell SA, Dement WC. Narcolepsy syndromes: antecedent, contiguous, and concomitant nocturnal sleep disordering and deprivation. Psychophysiology 1968; 4(3):398.
18. Mignot E, Hayduk R, Black J, et al. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. Sleep 1997; 20(11):1012–1020.
19. Browman CP, Gujavarty KS, Yolles SF, et al. Forty-eight hour polysomnographic evaluation of narcolepsy. Sleep 1986; 9(1 Pt 2):183–188.
20. Mayer G, Lattermann A, Mueller-Eckhardt G, et al. Segregation of HLA genes in multicase narcolepsy families. J Sleep Res 1998; 7(2):127–133.
21. Sturzenegger C, Bassetti CL. The clinical spectrum of narcolepsy with cataplexy: a reappraisal. J Sleep Res 2004; 13(4):395–406.

22. Dahlitz M, Parkes JD. Sleep paralysis. Lancet 1993; 341(8842):406–407.
23. Okun ML, Lin, L, Pelin Z, et al. Clinical aspects of narcolepsy-cataplexy across ethnic groups. Sleep 2002; 25(1):27–35.
24. Takeuchi T, Miyasita A, Sasaki Y, et al. Isolated sleep paralysis elicited by sleep interruption. Sleep 1992; 15(3):217–225.
25. Young T, Palta M, Dempsey J, et al. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328(17):1230–1235.
26. Bassetti C, Gugger M, Bischof M, et al. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin 1(orexin A). Sleep Medicine 2003; 4(1):7–12.
27. Hartse KM, Zorick FJ, Sicklesteel JM, et al. Isolated cataplexy: a familial study. Henry Ford Hosp Med J 1988; 36(1):24–27.
28. Roth B, Bruhova S, Berkova L. Familial sleep paralysis. Schweiz Arch Neurol Neurochir Psychiatr 1968; 102(2):321–330.
29. Carskadon MA, Dement WC, Mitler MM, et al. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep 1986; 9(4):519–524.
30. Rechtschaffen A, Kales A. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects, Washington, DC. U.S. Government Printing Office, 1968. (NIH publication no. 204.)
31. Mignot E, Young T, Lin L, et al. Nocturnal sleep and daytime sleepiness in normal subjects with HLA DQB1*0602. Sleep 1999; 22(3):347–352.
32. Anic-Labat S, Guilleminault C, Kraemer HC, et al. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. Sleep 1999; 22(1):77–87.
33. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14(6):540–545.
34. Mikulincer M, Babkoff H, Caspy T. The effects of 72 hours of sleep loss on psychological variables. British Journal of Psychology 1989; 80(pt 2):145–162.
35. Buzzi G, Cirignotta F. Isolated sleep paralysis: a web survey. Sleep Res Online, 2000; 3(2):61–66.
36. Maury MA. Des hallucinations hypnagogiques, ou des erreurs des sens dans l'état intermédiaire entre la veille et le sommeil. Annales Médico-Psychologiques 1848; II:26–40.

16
Neurophysiology of Cataplexy and Cataplexy-Like Phenomena

SEBASTIAAN OVEREEM
Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen and Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

GERT JAN LAMMERS
Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

BASTIAAN R. BLOEM
Department of Neurology, Radboud University, Nijmegen Medical Center, Nijmegen, the Netherlands

J. GERT VAN DIJK
Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

I. Introduction

Of the various symptoms of narcolepsy, cataplexy is the only specific one (1). Furthermore, the presence of cataplexy is a very specific indicator for hypocretin deficiency; in narcolepsy without cataplexy hypocretin is still present in most cases (2).

Cataplexy ("to strike down") is a physically and socially disabling symptom that may force patients to avoid potentially emotional situations. Fortunately, it can usually be treated to such good effect that attacks are almost eliminated, often at the price of side effects (1). This high efficacy contrasts sharply with the treatment of excessive daytime sleepiness, which almost always persists to a bothersome degree. In view of its importance for the diagnosis of narcolepsy and because it can be treated successfully, cataplexy must be identified correctly. Unfortunately, no diagnostic tool is available, and the diagnosis depends completely on history taking (1,3).

Cataplexy is a puzzling phenomenon. The combination of a sudden paralysis with preserved consciousness, triggered by emotions, fascinates physicians and laymen alike. Not surprisingly, a host of different underlying mechanisms have been proposed, including several psychoanalytical ones. Psychological explanations have now all been replaced by somatic ones, but the true pathophysiology of cataplexy largely remains enigmatic. Considerable knowledge, especially pharmacological, has been gathered from studies using the canine narcolepsy model (4). Experiments in human narcoleptics are still scarce. The discovery of the hypocretin/orexin system and its pivotal role in the

Figure 1 The expression "to be weak with laughter" also exists in the Chinese language. Printed here are two examples in colloquial Cantonese. Top line: "*laughing will lead to weakness of lower limbs*." Bottom line: "*laughing will lead to falling onto ground*."

pathophysiology of narcolepsy has not yet resulted in an increased insight in the neuronal mechanisms leading to cataplexy.

Several cataplexy-like phenomena can be found in man as well as in the animal kingdom. A prime human example is the universal experience of being "weak with laughter" (Fig. 1), which can be associated with a buckling feeling in the knees, sometimes even leading to falls (S. Overeem, personal observation).

In this chapter, we first discuss some relevant clinical features of cataplexy and briefly review neuroanatomical and neuropharmacological data obtained from animal models. We then describe current knowledge on the neurophysiology of cataplexy, primarily focusing on human studies. In vivo neurophysiological studies in narcoleptic patients may serve scientific as well as clinical diagnostic purposes. Cataplexy-like phenomena in animals will be discussed where they are relevant for the discussion at hand.

II. Clinical Aspects

A. Clinical Features During Cataplexy

Cataplexy consists of a sudden and bilateral loss of muscle tone in response to emotional stimuli (1,3). All skeletal muscles may be involved, except for those subserving ocular movements and respiration. During a complete attack, patients slump to the ground, fully conscious but unable to respond. Curiously, injuries during cataplectic attacks are uncommon. Most attacks do not involve a sudden and complete loss of control, but remain incomplete or evolve slowly enough to allow patients to break their fall. During complete attacks patients may indeed stagger and grasp for support, showing a gradual increase in severity. Partial attacks occur more frequently than complete ones. Two regions of the body are predominantly involved in partial attacks: the knees may give way, and involvement of the head and neck is visible as sagging of the jaw, inclination of the head and a slurred speech. The loss of control is often not continuous but intermittent, which is apparent as jerkiness of movements or grimacing. After a short period, lasting from several seconds to a few minutes, attacks stop rather abruptly, and patients can resume their activities.

B. Emotional Triggers

Cataplectic attacks are triggered by strong emotions, including humor, anger and surprise (5). Other frequent triggers are: when feeling tickled, attempts at repartee and, less often, unexpected meetings with acquaintances (1). It is interesting to note that laughter is mentioned as the most effective trigger throughout the literature, although strictly speaking it is not an emotion but a motor behavior. In daily life, most instances of laughter serve a social role and are not elicited by humor or mirth. Similarly, when patients describe the unexpected meeting of an acquaintance evoking cataplexy, the trigger is probably the emotion of surprise raised by the encounter rather than the encounter itself. Each patient experiences an individual sensitivity for the various emotions that provoke attacks. Attacks without any detectable trigger occur only rarely.

Although emotions are very important triggers, the circumstances and 'state' of the patient are also important (1). Characteristically, attacks cannot be provoked during medical consultation or in laboratory situations. It seems that a certain intimacy or a relaxed state is needed to lower the threshold for attacks to occur in company. Other circumstances that lower the attack threshold are a strong feeling of sleepiness and sleep deprivation. Most patients learn 'tricks' after several years to prevent or abort the attacks. Examples of such tricks are leaning against a wall, or the voluntary contraction of muscles that are not yet involved. Narcoleptics with severe cataplexy tend to avoid potential emotional situations, causing them to withdraw in part from social life.

III. Neurobiology of Cataplexy

The neurobiology of cataplexy, on a neuroanatomical and neuropharmacological level, has been discussed elsewhere in detail (for references, see (3,6,7)). Some basic mechanisms are discussed in this paragraph.

A. Neurochemistry and Neuroanatomy of Cataplexy

The neurochemical basis of cataplexy is complex, with pharmacological studies indicating the involvement of several neurotransmitter systems (see below). The canine model for narcolepsy has been invaluable to elucidate the role of the various brain regions and their interaction in the development of cataplexy (4). Several neuronal populations in the pontine brainstem are crucial for the generation of cataplexy. Based on extensive neuropharmacological and neurochemical experiments, Nishino et al. developed a model for the control of cataplexy in which both monoaminergic and cholinergic systems in the brainstem play key roles (4). In short, cataplexy is aggravated by cholinergic activation and by deactivation of monoaminergic systems, most importantly adrenergic ones. Brain regions outside the brainstem are also involved; using local injections cholinoceptive sites in the basal forebrain/anterior hypothalamus of narcoleptic dogs were shown to be very important in the modulation of cataplexy (8). Using microelectrodes, the electrical activity of a host of brain regions has been measured during cataplexy in narcoleptic canines. These studies showed that during cataplexy, several regions are activated that are known to be involved in the generation of REM sleep atonia (6). However, there are slight differences. For

example, neurons in the locus coeruleus are silent during both REM sleep and cataplexy, but neurons in the dorsal raphe, which are silent during REM sleep, are active during cataplexy (9).

The neurobiological substrate of the emotional trigger of cataplexy remains a mystery, and deserves further study. Emotional triggers are not exclusive to human narcolepsy. For example, attacks in narcoleptic dogs can occur on seeing a tasty morsel of food. Amygdala neurons often fire just before and during an episode of cataplexy, suggesting that they may help trigger the response (12).

B. The REM Sleep Dissociation Hypothesis

Extraocular and respiratory muscles remain functional during cataplexy. This resembles the situation during REM sleep, during which breathing continues and ocular movements occur despite the inhibition of the other skeletal musculature (6). This resemblance, together with the finding that Sleep Onset REM Periods (SOREMPs) are closely associated with narcolepsy, gave rise to the so-called REM-sleep dissociation theory to explain cataplexy (see (7)). This hypothesis portrays cataplexy, hypnagogic hallucinations and sleep paralysis as partial features of REM sleep occurring during the waking state. Cataplexy represents the inadvertent expression of REM sleep atonia in this view (3).

Although the REM sleep dissociation hypothesis has been generally accepted, there are several other arguments against this theory. For example, the ultradian rhythm of REM sleep is completely intact in narcoleptic dogs (11). Furthermore, the REM-dissociation theory does not explain why cataplexy is typically brought on by emotions.

C. The Role of the Hypocretin System

Another area of uncertainty concerns the link between hypocretin deficiency and the occurrence of cataplexy. The relationship may be causal, in view of the very strong linkage between the presence of cataplexy and the absence of CSF hypocretin-1 in humans (2). Recent studies suggest that hypocretin normally acts at multiple levels to suppress the unwanted onset of atonia. On the one hand, the hypocretins seem to inhibit various neuronal mechanisms involved in the generation of REM sleep atonia (13). On the other hand, hypocretin may influence muscle tone through direct projections onto spinal motoneurons (14). Interestingly, this influence of hypocretin on the activity of alpha motoneurons seems mediated through both pre- and postsynaptic mechanisms.

In conclusion, the nature of cataplexy is largely unknown. In the following section we discuss how clinical neurophysiological studies may help unravel cataplexy in humans. In humans, emotional triggers are easier to study than in animals. Another important goal will be to further dissect the pathways that mediate muscle atonia in cataplexy, and to investigate whether pre- or postsynaptic mechanisms play a role. This will not only show whether the REM sleep dissociation theory is correct or not, but can also guide new therapeutic approaches for cataplexy, as post- and presynaptic inhibitory pathways make use of different neurotransmitters.

IV. Neurophysiological Investigations During Cataplexy

There are only few reports on neurophysiological studies during attacks of cataplexy in man, presumably because of the difficulties in eliciting cataplexy in the laboratory. The classical study was performed by Guilleminault et al., who combined EEG, EMG and H-reflex recordings together with neurological examinations in a number of patients during multiple cataplectic attacks (15). Most other studies only used some of these techniques.

Neurological examination during cataplexy shows a flaccid paralysis of the affected muscles (3). In addition, tendon reflexes are lost; even in muscles not affected during an attack (16). In some cases, an extensor plantar response is found.

A. EEG, EMG and Reflex Recordings

Consciousness is preserved during cataplexy. Accordingly, EEG recordings showed a pattern of normal wakefulness during cataplexy (15,17). In the course of longer cataplectic attacks, the EEG may sometimes show features of REM sleep and patients may later report dreaming, making it difficult to distinguish cataplexy from (REM) sleep (3,18).

During complete cataplectic attacks, EMG activity is abruptly diminished in both agonist and antagonist extremity muscles (15,17). Partial attacks are associated with a more localized loss of EMG activity, for example only in jaw or neck muscles. However, even in such cases, there is a diminution of EMG activity in other (clinically not involved) muscles. Rubboli and colleagues studied the time course of muscle weakness in a single patient, standing upright (17). They found that the polygraphic EMG pattern was quite stereotyped. They identified three phases, i.e., initial, falling and an atonic phase. During the initial phase, there was buckling of the knees accompanied by a few isolated jerking body movements. In the falling phase the jaw sagged, the head and trunk bent over after which the patient fell. There were rhythmic "rebounds" of muscle tone during this phase, resulting in jerking movements (Fig. 2). Finally, when the patient was lying on the ground, the atonic phase appeared, characterized by complete immobility (Fig. 2). In our experience, muscle twitches and jerking movements described in various studies are very typical for cataplexy, and may even have some diagnostic value.

It is generally thought that cataplexy is mediated by a direct inhibition of motoneuron cell bodies at the spinal level (postsynaptic inhibition) (6). This would explain why tendon reflexes are depressed or abolished during cataplexy, but does not explain why reflexes disappear in muscles that are not paralyzed. In order to further study the spinal mechanisms involved, Guilleminault et al recorded H-reflexes during cataplexy in 5 subjects (15). The H-reflex is an electrically elicited equivalent of the monosynaptic muscle stretch reflex (Fig. 3a). Its amplitude is influenced by supraspinal influences, for example descending pathways that alter the excitability of spinal alpha-motoneurons. The H-reflex was completely abolished during generalized cataplectic attacks (15). In partial attacks involving only jaw or neck muscles, the H-reflex in leg muscles disappeared only in the beginning of the attack, after which it reappeared, although with a smaller amplitude.

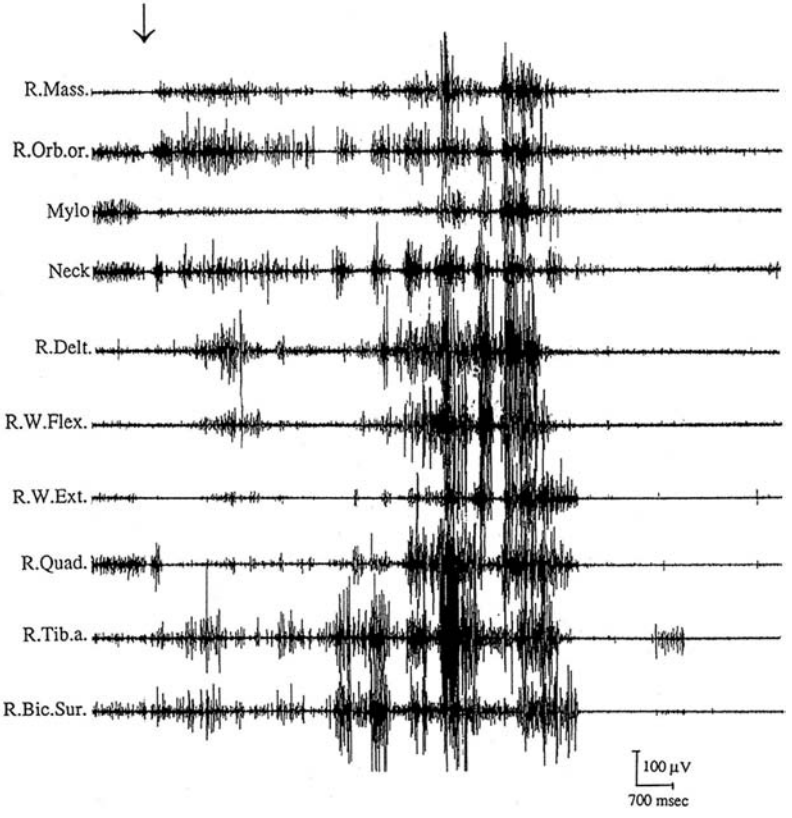


Figure 2 EMG registration during a laughter-induced complete cataplectic attack in a standing patient. The figure shows a detail of the polygraphic pattern of the falling phase (indicated by the horizontal dashed line). The arrow indicates a phasic postural lapse, preceding by few seconds the appearance of rhythmic suppressions and rebounds of EMG activity, involving synchronously all recorded muscles, associated with the gradual fall to the ground of the patient. Reprinted from: Rubboli et al. (17). *Abbreviations*: Mass: masseter; Orb.or: orbicularis oris; Mylo: mylohyoideus; Neck: cervical portion of the trapezius; Delt: deltoid; W.Flex: wrist flexor; W.Ext: wrist extensor; Quadr: quadriceps; Tib.A: tibialis anterior; Bic Sur: femoral biceps.

B. Transcranial Magnetic Stimulation

Another means of studying the motor system during cataplexy is transcranial magnetic stimulation (TMS). Using TMS, the motor cortex is stimulated with a magnetic coil held above the scalp. TMS activates pyramidal neurons directly or indirectly and ultimately excites alpha motoneurons in the spinal cord, resulting in muscle contraction. Only one case report described TMS results during a cataplectic attack (19). Interestingly, it was found that the response amplitudes in all muscles studied remained unaltered during cataplexy. The authors explained this through assuming that cortical excitability must have increased to counter decreased alpha motoneuron excitability. An alternative explanation is that cataplexy is not due to a purely postsynaptic

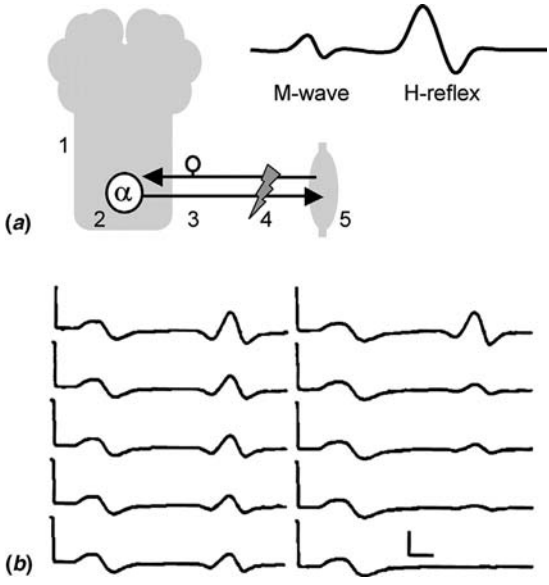


Figure 3 (***a***) Schematic representation of the H-reflex pathway, which involves stimulation of a peripheral nerve (indicated by the lightning symbol), leading first to a M-wave through motor nerve excitation, and to the H-reflex itself, resulting from sensory nerve excitation, a synapse with the alpha motoneuron, and muscle contraction. 1: spinal cord. 2: alpha-motoneuron. 3: peripheral nerve (typically tibial nerve). 4: stimulation site (typically popliteal fossa). 5: target muscle (typically soleus muscle). (***b***) Example of H-reflex changes during emotive stimuli, in a healthy control subject. Both panels show the five successive H-reflexes recorded during a slide. The right panel shows a large decrease measured during laughter, and the left panel shows a lesser decrease obtained when the subject did not laugh. Note that the small inframaximal M-wave remains stable, signifying a stable recording.

inhibition of motoneurons, as responses to TMS would then have to be diminished (for a more detailed discussion, see Ref. 20).

C. Autonomic Changes

In addition to alterations in muscle tone and reflex activity, cataplexy seems to be associated with autonomic changes. Studies in humans reported a decrease in blood pressure at the onset of cataplexy, with a compensatory tachycardia (3). A polygraphic study in a single patient demonstrated that the majority of cataplectic attacks are accompanied by bradycardia after an initial increase in heart rate (17).

D. Implications for the Pathophysiology of Cataplexy

The concept of cataplexy as a dissociated manifestation of REM-sleep atonia was based in part on the observation of a disappearing H-reflex during cataplexy, as the H-reflex is absent during REM-sleep (3,6). As REM-sleep atonia is mediated by a direct post-synaptic inhibition of alpha-motoneurons (10), it was hypothesized that cataplexy must be due to the same mechanism. However, earlier hypothesis had in fact suggested

the possibility of presynaptic inhibitory mechanisms during REM sleep (21). The possibility that either pre- or postsynaptic inhibition could explain cataplexy had been acknowledged in early papers (15), but the consensus shifted towards postsynaptic inhibition as the main mechanism in cataplexy (3), in parallel with the development of the REM-sleep dissociation theory. There is no factual evidence to favor postsynaptic inhibition, however. On the contrary, the limited TMS data available on human cataplexy suggest the opposite (see above). The neurophysiological findings during cataplexy (atonia, abolished tendon-, and H-reflexes) can be explained convincingly by pre-synaptic inhibition of afferent neurons. If this is proven, this would argue against the hypothesis that cataplexy is a direct analogue of REM-sleep atonia (20).

V. Neurophysiological Studies Between Cataplectic Attacks

The difficulty of inducing cataplexy under laboratory circumstances severely limits the study of such attacks. Another approach is to measure cataplectic patients *outside* attacks, in order to measure their propensity to develop cataplexy. This allows larger numbers of subjects to be studied and may, if abnormalities are found, even result in a diagnostic technique. Measuring treatment effects may also be possible.

Various tests have been applied to cataplectic subjects outside attacks. Masseter- and blink reflexes, as well as electro-oculography were shown to be unsuitable (22). Studies on evoked potentials were inconclusive (22).

A. H-Reflex Studies

Given the knowledge on H-reflex changes *during* cataplexy, we studied several H-reflex parameters in narcoleptic patients at rest, and compared them to control values (23). Mean H-amplitudes, as well as H/M ratios (correcting the H-amplitude for differences in muscle mass) did not differ between patients and controls. We then measured continuous H-reflexes during various visually presented emotive stimuli. We intended to apply some "emotional stimulation", and did expect to evoke actual cataplexy. The H-reflex greatly diminished and sometimes even disappeared when patients laughed out loud. This effect was as strong in patients as in healthy controls (Fig. 3b) (23,24). These findings showed that H-reflex alterations during cataplexy (15) must be interpreted with caution: as cataplexy was elicited by laughter in these studies, H-reflex alterations may in part have been secondary to laughter itself, and not related to cataplexy directly. There are several arguments that do point to a role for cataplexy: H-reflex suppression during laughter was more pronounced in more severely affected patients, and less pronounced in patients using anti-cataplectic medication (23).

B. Startle Reflexes

In hereditary hyperekplexia (startle disease), patients react to a sudden auditory stimulus with an excessive startle response and an increase in muscle tone. Based on the contrast between cataplexy and hyperekplexia, we studied startle reflexes in patients with narcolepsy (23). We hypothesized that hyperekplexia and cataplexy represent

two opposite extremes of a spectrum of pathological regulation of muscle tone. Surprisingly, we found a clear *increase* in the magnitude of the startle response of cataplectic subjects as compared to controls. The exaggerated startle response may be due to brainstem abnormalities influencing the startle reflex, or be secondary to an altered alpha-adrenergic "tone" in narcolepsy. Cataplexy is reduced by alpha-adrenergic stimulation (4). Consequently, narcoleptics may have an increased alpha-adrenergic tone to partial compensate for their propensity for cataplexy, ultimately leading to an increased startle response as a "side effect."

VI. Cataplexy-Like Phenomena

Several phenomena in man and animals resemble cataplexy in some way. The feeling of being weak with laughter has been mentioned. Tonic immobility is an animal response pattern that has some striking similarities with cataplexy, in that it is one of the few motor responses directly elicited by emotions. Studying these phenomena may help to understand the nature of cataplexy.

A. Weak with Laughter

In humans, motor control during laughter is altered profoundly. This is not only measurable neurophysiologically, but also translates to a subjective feeling of weakness (Fig. 1) (24). The question arises whether cataplexy and the physiological "weakness with laughter" are completely distinct phenomena, or whether they form a continuum (25).

In any case, motor inhibition during laughter appears to differ fundamentally from that during REM-sleep. During hearty laughter in healthy subjects, the H-reflex was suppressed just as in REM sleep (Fig. 3b). However, simultaneously applied TMS showed that motor responses were unaltered (20). This combination argues against direct inhibition of alpha motoneurons. A more likely alternative is that the H-reflex alterations must be explained by presynaptic inhibition of sensory afferents (20). Interestingly, the only TMS study performed during cataplexy found preserved muscle responses to magnetic stimulation (19).

B. Tonic Immobility

We recently compared the animal behavior pattern called "tonic immobility" (TI) to cataplexy (7). TI is characterized by severe motor inhibition when an animal faces grave danger, such as the approach of a predator (26). The nomenclature of this reaction pattern is highly confusing, with numerous terms being used, such as animal hypnosis, immobility reflex, "Totstellreflex," feigning death, playing dead and fright paralysis. During TI, animals are fully conscious, and actually "keep a check" on their surroundings.

Surprisingly few studies evaluated muscle tone during TI. Klemm described a "waxy flexibility" in which there may be muscle atonia (27). In addition to motor inhibition, TI is also associated with some autonomic alterations, including blood pressure decreases and bradycardia. Based on these features, we proposed that TI might serve as a model for cataplexy (7).

VII. Neurophysiological Methods as a Diagnostic Aid?

It is important to reliably identify cataplexy both for diagnostic and therapeutic reasons, but this proves difficult. Several disorders that can mimic cataplexy need to be distinguished from it, for example syncope and epilepsy. History taking is currently the only diagnostic method, but this poses problems: partial attacks can be so subtle that they are often only recognized by experienced observers, such as partners or specialized physicians. Even patients themselves may fail to interpret this phenomenon as abnormal.

The importance of diagnosing cataplexy accurately has led to attempts to develop an objective diagnostic test to measure or quantify the propensity for cataplexy. It may be possible to detect a propensity to develop cataplexy, using neurophysiological measurements at rest, without trying to elicit a cataplectic attack. Alternatively, one can attempt to provoke cataplexy using emotional triggers, performing tests when an attack ensues. Finally, it may be possible to *induce* cataplexy in cataplexy-prone subjects, pharmacologically or otherwise.

A. Probing a Propensity for Cataplexy

As described previously, brain-stem reflexes were unaltered in narcoleptic patients, as were H-reflexes at rest. Using emotional stimulation, it proved possible to evoke an H-reflex suppression in narcoleptic subjects, but this was also the case in control subjects. The finding that narcoleptics have an exaggerated startle response may be useful as a diagnostic aid. However, for this purpose, it is necessary to conduct a large prospective study in narcoleptics and controls, to determine the diagnostic value of this technique in individual cases.

B. Provoking Cataplexy

Dyken et al. proposed to diagnose cataplexy by provoking cataplexy through telling jokes, and concomitantly perform a polysomnographic recording (18). They were able to elicit at least one cataplectic attack in four highly selected patients, one of which had recently stopped taking medication. In our view polysomnography does not help the diagnosis when a cataplectic attack can be witnessed. Assessing muscle tone and checking for preserved consciousness, e.g., by asking the patients to memorize some words, will suffice. Moreover, given the effects of laughter on the H-reflex in healthy controls, absent tendon reflexes in laughter-evoked-spells is not specific for narcolepsy, although preserved reflexes during an attack argue against cataplexy.

Krahn et al. tried to standardize the emotional triggers that can be used to elicit cataplexy (16). They compiled a videotape with a series of humorous scenes, and showed it to nine narcoleptic patients, while simultaneously recording EMG, EEG and EOG, and testing the quadriceps reflex in four patients during an attack. An attack was only evoked in five out of the nine patients. In three patients this occurred in response to a different trigger than the standard video intended to be used. Furthermore, six patients with a clinical suspicion of having narcolepsy/cataplexy were excluded from the study because of a negative MSLT. This is unfortunate, as an objective cataplexy test would be particularly appropriate for this patient category.

In conclusion there is no practical way of eliciting cataplexy reliably. If a cataplectic attack is witnessed, this greatly aids the diagnosis. Future attempts to standardize a "triggering protocol" should include an unselected, prospective patient cohort and, importantly, healthy control subjects as well.

C. Inducing Cataplexy

There is only one study in which neurophysiological techniques were used to induce cataplexy. Hungs et al used repetitive TMS (rTMS) in three patients who contracted their first dorsal interosseous muscle (FDI). They found that rTMS induced a short-lasting interruption of voluntary EMG activity (28). This muscle atonia in response to rTMS was not observed in eight healthy controls. Unfortunately, the stimulus parameters used for the repetitive rTMS (intensity 110% from resting motor threshold, 20 Hz for two seconds) are in violation with generally accepted safety regulations for rTMS (29). Furthermore, the patients had all been acutely withdrawn from anti-cataplectic medication, which may have affected the motor system profoundly. We tried to replicate their findings using rTMS parameters within safety margins on unmedicated patients, but failed to provoke cataplexy (unpublished data).

A potentially more effective approach will be to pharmacologically aggravate the tendency of developing cataplexy in narcoleptic patients, so that attacks can be observed and studied. The alpha-1 antagonist prazosine for example may be suitable for this purpose (30).

VIII. Future Perspectives

Cataplexy remains a mysterious and puzzling symptom. The discovery of hypocretin defects represented a breakthrough in the pathogenesis of narcolepsy, but our understanding of the pathophysiology of cataplexy has not improved appreciably, if at all.

The canine narcolepsy model remains the only one in which cataplexy can be provoked and studied reliably. Hypocretin-deficient transgenic rodent models apparently display cataplexy-like behavior, but this behavior has not yet been characterized with precision, and no knowledge regarding motor physiology during attacks is available.

Alternative pathophysiological frameworks besides the REM sleep dissociation theory should be studied (11,25). We recently proposed that cataplexy might be an atavistic expression of TI, based on a number of striking similarities of the two conditions (7). This hypothesis can be studied, starting with a more detailed elucidation of motor function during TI, and by evoking TI in hypocretin-deficient rodents.

Much remains to be learned from studying human cataplexy. Hopefully, such research efforts will ultimately lead to a more sensitive and precise diagnosis of cataplexy, and more effective and better-tolerated treatments for this disabling symptom.

Acknowledgments

Sebastiaan Overeem is supported by Veni Grant 016.056.103 from the Netherlands Organisation for Scientific Research. We would like to thank Dr. R Sybesma

(Department of Chinese Languages and Cultures, Leiden University, The Netherlands) for providing us with Figure 1, and Dr. H. Droogleever-Fortuyn (Department of Psychiatry, University Medical Center Nijmegen, The Netherlands) for helpful discussion.

References

1. Overeem S, Mignot E, van Dijk JG, Lammers GJ. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. J Clin Neurophysiol 2001; 18:78–105.
2. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
3. Guilleminault C, Gelb M. Clinical aspects and features of cataplexy. Adv Neurol 1995; 67:65–77.
4. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol 1997; 52:27–78.
5. Anic-Labat S, Guilleminault C, Kraemer HC, Meehan J, Arrigoni J, Mignot E. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. Sleep 1999; 22:77–87.
6. Hishikawa Y, Shimizu T. Physiology of REM sleep, cataplexy, and sleep paralysis. Adv Neurol 1995; 67:245–271.
7. Overeem S, Lammers GJ, van Dijk JG. Cataplexy: 'tonic immobility' or 'REM-sleep atonia'? Sleep Medicine 2002; 3:471–477.
8. Nishino S, Tafti M, Reid MS, Shelton J, Siegel JM, Dement WC, Mignot E. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. J Neurosci 1995; 15:4806–4814.
9. Wu MF, John J, Boehmer LN, Yau D, Nguyen GB, Siegel JM. Activity of dorsal raphe cells across the sleep-waking cycle and during cataplexy in narcoleptic dogs. J Physiol 2004; 554:202–215.
10. Chase MH, Morales FR. The atonia and myoclonia of active (REM) sleep. Annu Rev Psychol 1990; 41:557–584.
11. Nishino S, Riehl J, Hong J, Kwan M, Reid MS, Mignot E. Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. Neurosci Res 2000; 38:437–446.
12. Gulyani S, Wu MF, Nienhuis R, John J, Siegel JM. Cataplexy-related neurons in the amygdala of the narcoleptic dog. Neuroscience 2002; 112:355–365.
13. Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci USA 1999; 96:10911–10916.
14. Yamuy J, Fung SJ, Xi M, Chase MH. Hypocretinergic control of spinal cord motoneurons. J Neurosci 2004; 24:5336–5345.
15. Guilleminault C, Wilson RA, Dement WC. A study on cataplexy. Arch Neurol 1974; 31:255–261.
16. Krahn LE, Boeve BF, Olson EJ, Herold DL, Silber MH. A standardized test for cataplexy. Sleep Med 2000; 1:125–130.
17. Rubboli G, d'Orsi G, Zaniboni A, Gardella E, Zamagni M, Rizzi R, Meletti S, Valzania F, Tropeani A, Tassinari CA. A video-polygraphic analysis of the cataplectic attack. Clin Neurophysiol 2000; 111 (Suppl 2):S120–S128.
18. Dyken ME, Yamada T, Lin-Dyken DC, Seaba P, Yeh M. Diagnosing narcolepsy through the simultaneous clinical and electrophysiologic analysis of cataplexy. Arch Neurol 1996; 53:456–460.
19. Rosler KM, Nirkko AC, Rihs F, Hess CW. Motor-evoked responses to transcranial brain stimulation persist during cataplexy: a case report. Sleep 1994; 17:168–171.
20. Overeem S, Reijntjes R, Huyser W, Lammers GJ, van Dijk JG. Corticospinal excitability during laughter: implications for cataplexy and the comparison with REM sleep atonia. J Sleep Res 2004; 13:257–264.
21. Baldissera F, Cesa-Bianchi M, Mancia M. Phasic events indicating presynaptic inhibition of primary afferents to the spinal cord during desynchronized sleep. J Neurophysiol 1966; 29:871–887.
22. Marx JJ, Urban PP, Hopf HC, Grun B, Querings K, Dahmen N. Electrophysiological brain stem investigations in idiopathic narcolepsy. J Neurol 1998; 245:537–541.

23. Lammers GJ, Overeem S, Tijssen MA, van Dijk JG. Effects of startle and laughter in cataplectic subjects: a neurophysiological study between attacks. Clin Neurophysiol 2000; 111:1276–1281.
24. Overeem S, Lammers GJ, van Dijk JG. Weak with laughter. Lancet 1999; 354:838.
25. Bassetti C. Editorial. Cataplexy: 'REM-atonia or tonic immobility'? Sleep Med 2002; 3:465–466.
26. Klemm WR. Neurophysiologic studies of the immobility reflex ("animal hypnosis"). Neurosci Res (NY) 1971; 4:165–212.
27. Carli G. Depression of somatic reflexes during rabbit hypnosis. Brain Res 1968; 11:453–456.
28. Hungs M, Mottaghy FM, Sparing R, Zuchner S, Boroojerdi B, Topper R. RTMS induces brief events of muscle atonia in patients with narcolepsy. Sleep 2000; 23:1099–1104.
29. Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. Electroencephalogr Clin Neurophysiol 1998; 108:1–16.
30. Aldrich MS, Rogers AE. Exacerbation of human cataplexy by prazosin. Sleep 1989; 12:254–256.

17
Abnormal Motor Activity During Sleep in Narcolepsy

JACQUES MONTPLAISIR, SHIRLEY WHITTOM, and SYLVIE ROMPRÉ
Centre d'Étude du Sommeil, Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada

THIEN DANG-VU
Department of Neurology, Université de Liége, Cyclotron Research Center, Liege, Belgium

YVES DAUVILLIERS
CHU Montpellier, Unité de Sommeil Hôpital Gui de Chauliac, Montpellier, France

There are several types of abnormal motor activity noted during sleep in patients with narcolepsy including periodic leg movements in sleep (PLMS), periodic leg movements while awake (PLMW) and REM sleep behavior disorder (RBD). This chapter will focus mostly on PLMS but other types of abnormal motor activity will be briefly discussed.

I. Periodic Leg Movements in Sleep (PLMS)

A. Introduction

Periodic leg movements in sleep (PLMS) are characterized by rhythmical extensions of the big toe and dorsiflexion of the ankle with occasional flexion of the knee and hip. Methods for recording and scoring PLMS were developed by Coleman (1). According to the standard criteria, PLMS are scored only if they are part of a series of four or more consecutive movements lasting 0.5 to 5 seconds with an inter-movement interval of 4 to 90 seconds. A PLMS index (number of PLMS per hour of sleep) greater than 5 for the entire night of sleep is considered pathological. Almost everything that we know on PLMS comes from the study of patients with the Restless legs syndrome (RLS). In these patients, the number of PLMS varies from night to night especially in individuals with less severe sleep complaints. PLMS also cluster into episodes each of which lasts several minutes or even hours. In general, these episodes are more numerous in the first third of the night but they can also recur throughout the entire sleep period. PLMS are often associated with EEG signs of arousal. These arousals may be of short duration insufficient for scoring an epoch of wakefulness and are therefore named EEG arousals or micro-arousals (MA). Recently, more attention has been paid to other signs of physiological activation associated with PLMS (2). Regardless of the presence of MA, almost every PLMS are associated with an EKG response, namely a tachycardia (decrease of RR intervals from 5 to 10 beats) followed by a bradycardia. The entire event lasts approximately 20 seconds.

If PLMS were first documented in patients with the Restless legs syndrome (RLS) (3) they also occur in a wide range of sleep disorders including narcolepsy, REM sleep behavior disorder, obstructive sleep apnea, insomnia, and hypersomnia (4). PLMS were also reported in subjects without any sleep complaint and while they are rare in young individuals, they are relatively common in the elderly. In a recent study of 70 normal middle-aged individuals, aged 40 to 60 (mean 51.3 $\pm$ 5.0), 52% of men and 28% of women had a PLMS index greater than 5 (5). In that study, there was no difference in sleep architecture between normal subjects with and without PLMS. When PLMS are seen in patients who complain of primary sleep onset or sleep maintenance insomnia or of primary hypersomnia, they are referred to as PLM disorder. The basic assumption is that PLMS are responsible for the non-restorative sleep and daytime somnolence reported by these patients. However, in a study performed in our laboratory (6), no correlation was found between the PLMS index, sleep efficiency and daytime vigilance in a population of 34 non-narcoleptic and nonapneic hypersomnia patients with PLMS. So, although some studies suggested that PLMS may be associated with sleep-wake complaints, a majority have concluded that PLMS seen in patients with insomnia or hypersomnia or normal controls have little impact on nocturnal sleep and daytime vigilance.

A recent study looked at the prevalence of PLMS in five groups of 20 subjects, namely, controls subjects, patients complaining of insomnia, of hypersomnia, of narcolepsy and of RLS. The five groups were matched for age and gender. Results of that study showed that although a large number of insomniacs, hypersomniacs and normal controls have a PLMS index greater than five, the mean PLMS index was similar in these three groups. On the other hand, PLMS indices were higher in patients with narcolepsy or RLS. Another study (7) looked at PLMS in a population of patients with REM sleep behaviour disorder (RBD) and found that 80% of RBD patients had a PLMS index $\geq$ 5. These results are similar to those obtained in a group of gender and age-matched normal controls.

B. Periodic Leg Movements in Patients with Narcolepsy

Elevated PLMS indices were frequently reported in patients with narcolepsy (for a review see 4). Recently, we looked at the prevalence and the characteristics of PLMS in a group of 170 patients with narcolepsy and compared these results with those of 50 age- and gender-matched normal controls (Montplaisir et al. unpublished data).

The inclusion criterion for narcoleptic patients was the presence of both excessive daytime sleepiness and cataplexy. All patients were HLA DR2 positive and all had at least one sleep-onset-REM-period (SOREMP) during the Multiple Sleep Latency Test (MSLT). The exclusion criteria for the narcoleptics were the presence of any other sleep disorders, other psychiatric or neurological disorders and the use of any treatment for narcolepsy or medication known to influence sleep or motor activity. The presence of the Sleep Apnea Syndrome (SAS) was ruled out on the basis of the polysomnographic recording; all subjects with an index of respiratory events (apnea + hypopnea) greater than 10 were excluded from the study. The 50 normal controls were matched for age and gender to the narcoleptic patients. The exclusion criteria for the normal controls were the same as for narcoleptics. In addition, none of the normal controls were

Table 1 PLMS and PLMW in Narcoleptics and Control Subjects

	Narcoleptics N = 170	Controls N = 50	P t-test
PSG age	46.1 ± 14.1	49.0 ± 8.8	ns
PLMS Index	21.5 ± 29.5	3.7 ± 6.1	0.001[b]
PLMW Index	37.7 ± 39.6	14.4 ± 16.8	0.002[b]
PLMS Index >5 (%)	68%	26%	0.00001[a]
PLMS Index >10 (%)	53%	12%	0.00001[a]

[a]Chi-square test.
[b]t-test performed on log transformed variable.

complaining about sleep or vigilance nor were they presenting any of the symptoms of narcolepsy.

Results presented in the Table 1 show that narcoleptic patients had more PLMS than control subjects (PLMS index = 21.5 vs. 3.7) and more narcoleptics (68%) than controls (26%) had a PLMS greater than 5. There were also more periodic leg movements during wakefulness (PLMW) in narcoleptics (PLMW index = 37.7 versus 14.4). When we compared narcoleptics with and without PLMS, we found that patients with PLMS were significantly older (51 vs. 41 years) and a strong correlation was found between PLMS index and age in patients with narcolepsy ($r = 0.44$, $p < 0.001$).

C. Functional Significance of PLMS in Narcolepsy

There were many differences in sleep architecture between narcoleptics with and without PLMS. However most of these differences were related to age. A correlation performed between PLMS on one hand and sleep variables, severity of cataplexy or results of the MSLT on the other hand, with age as a co-factor revealed that the presence of PLMS was positively correlated to PLMW index, micro-arousal index and stage 1 sleep percent. There was no significant correlation between PLMS, and severity of cataplexy, sleep latency or number of SOREMPs on the MSLT.

We also looked at autonomic changes associated with PLMS in narcolepsy and compared these changes to those obtained in patients with RLS. In RLS as in normal subjects, PLMS are associated with a tachycardia followed by a bradycardia, the sequence lasting approximately 20s (2). There was a marked decrease in heart rate change associated with PLMS in narcolepsy with a lower amplitude of tachycardia and the absence of subsequent bradycardia. Similar results were obtained previously in patients with RBD (7). These observations suggest the presence of an autonomic dysfunction in narcolepsy and RBD.

D. Physiopathology of PLMS

There are several evidences that dopamine (DA) plays a major role in the pathophysiology of PLMS. First, PLMS were reported in a large number of normal subjects with advancing age and there are several evidences from animal and human researches that D2 receptors decrease with aging. An increased prevalence of PLMS was also seen in association with specific sleep disorders, namely RLS, narcolepsy, and RBD. There are several evidences for DA mechanisms to be impaired in each of these

three conditions (for review see 4). On the other hand, a study of elderly schizophrenics (8) found a low prevalence of PLMS, suggesting that the increase of DA transmission in this population may be associated with the lower risk of developing PLMS.

The DA hypothesis of PLMS is also supported by pharmacological studies. It is well known that the treatment of the RLS with levodopa or with DA agonists pergolide, carbergoline, pramipexole and ropinirole not only suppressed symptoms of RLS in the waking state but also strongly suppressed PLMS (9–12). Similarly, one study (13) found that levodopa decreased PLMS in patients with narcolepsy. In that study, the PLMS index decreased from 21.3 to 9.9 after treatment with levodopa. A study of bromocriptine (14) also showed a marked decrease of PLMS index (from 43.4 to 15.9) in narcolepsy. None of these studies showed changes in sleep architecture in narcoleptic patients treated with DA agents.

Another set of evidences comes from brain imaging studies, conducted both in SPECT and in PET in patients with RLS-PLMS. Overall, these studies showed little or no change at the pre-synaptic level (15–17) and a small decrease in binding to post-synaptic D2 receptors both in patients with RLS-PLMS and patients with PLMS alone (16–18). One problem with brain imaging studies is that they were all conducted during the daytime when most of the patients are asymptomatic. Future studies should include daytime and nighttime PET or SPECT in patients with PLMS. Neuro-endocrine data are also congruent with the DA hypothesis of PLS at least in patients with RLS. Levodopa normally produces a suppression of prolactin and an increase of growth hormone secretion. A recent study by Borreguero et al. (19) showed that these effects of levodopa were markedly increased in RLS-PLMS patients at night compared to normal controls. This result was interpreted as an hypersensitivity of DA receptors at night in patients with RLS-PLMS. These authors also found a strong correlation between suppression of prolactin at night and the PLMS index. This result supports the DA hypothesis of PLMS and suggests the possibility that extra-striatal DA systems may be involved.

PLMS were also seen in narcoleptic canines (20). In dogs, PLMS are characterized by dorsi-flexion of the ankle lasting 0.5 to 1.5 s and recurring at intervals of 3 to 20 s. The conclusion of this study was that altered dopamine regulation in canine narcolepsy may play a critical role in both cataplexy and PLMS.

II. REM Sleep Behavior Disorder (RBD)

RBD has been reported in 42% of patients with narcolepsy (21). RBD is characterized by REM sleep without atonia. This form of state dissociation is opposite to cataplexy, characterized by atonia without REM sleep. There are several similarities between RBD and narcolepsy. Both conditions showed a high prevalence of PLMS (7). Also, autonomic dysfunction was found in both conditions in the form of reduced cardiac activation in response to PLMS or micro-arousals. Finally, in patients with Parkinson's disease with hallucinations, a strong association was found with RBD but also with polysomnographic features of narcolepsy (sleep onset REM periods), (22). All these observations suggest the possibility that RBD and narcolepsy may share common neurobiological deficits.

References

1. Coleman RM. Periodic movements in sleep (nocturnal myoclonus) and restless legs syndrome. In: Guilleminault C, ed, Sleeping and Waking Disorders: Indications and Techniques. Menlo Park California: Addison-Wesley, 1982:265–295.
2. Sforza E, Nicolas A, Lavigne G, Gosselin A, Petit D, Montplaisir J. EEG and cardiac activation during periodic leg movements in sleep: support for a hierarchy of arousal responses. Neurology 1999; 52(4):786–791.
3. Lugaresi E, Cirignotta F, Coccagna G, et al. Nocturnal myoclonus and restless legs syndrome. In: S Fahn et al. eds., Advances in Neurology, New York: Raven Press, 1986; 43:295–307.
4. Montplaisir J, Michaud M, Denesle R, Gosselin A. Periodic leg movements are not more prevalent in insomnia or hypersomnia but are specifically associated with sleep disorders involving a dopaminergic impairment. Sleep Med 2000; 1(2):163–167.
5. Frenette S, Paquet J, Montplaisir J, Carrier J. To be excluded or not: how periodic leg movement index influences polysomnographic selep in middle-aged subjects without sleep complaints? Sleep 27(abs suppl):A308 #689.
6. Nicolas A, Lespérance P, Montplaisir J. Is excessive daytime sleepiness with periodic leg movements during sleep a specific diagnostic category? Eur Neurol 1998; 40:22–26.
7. Fantini L, Michaud M, Gosselin N, Lavigne G, Montplaisir J. Periodic leg movements in REM sleep behavior disorder and related autonomic and EEG activation. Neurology 2002; 59(12):1889–1894.
8. Ancoli-Israel S, et al. Sleep-disordered breathing and periodic limb movements in sleep in older patients with schizophrenia. Biol Psychiatry. 1999; 45(11):1426–1432.
9. Wetter TC, Stiasny K, Winkelmann J, Buhlinger A, Brandenburg U, Penzel T, Medori R, Rubin M, Oertel WH, Trenkwalder C. A randomized controlled study of pergolide in patients with restless legs syndrome. Neurology 1999; 52(5):944–950.
10. Stiasny K, Wetter TC, Winkelmann J, et al. Long-term effects of pergolide in the treatment of restless legs syndrome. Neurology 2001; 56(10):1399–1402.
11. Montplaisir J, Nicolas A, Denesle R, Gomez-Mancilla B. RLS improved by Pramipexole: a double-blind randomized trial. Neurology 1999; 52(5):938–943.
12. Allen R, et al. Ropinirole decreases periodic leg movements and improves sleep parameters in patients with Restless legs syndrome. Sleep 2004; 5:907–914.
13. Boivin DB, Montplaisir J, Poirier G. The effects of L-dopa on periodic leg movements and sleep organization in narcolepsy. Clin Neuropharmacol 1989; 12(4):339–345.
14. Boivin DB, Lorrain D, Montplaisir J. The effects of bromocriptine on periodic limb movements in human narcolepsy. Neurology 1993; 43:2134–2136.
15. Eisensehr I, Wetter TC, Linke R, et al. Normal IPT and IBZM SPECT in drug-naive and levodopa-treated idiopathic restless legs syndrome. Neurology 2001; 57(7):1307–1309.
16. Michaud M, Soucy JP, Chabli A, Lavigne G, Montplaisir J. SPECT imaging of striatal pre- and postsynaptic dopaminergic status in restless legs syndrome. J Neurol 2002; 249:164–170.
17. Turjansky N, Lees A, Brooks DJ. Striatal dopaminergic function in restless legs syndrome: ^{18}F-dopa and ^{11}C-raclopride PET. Neurology 1999; 52(5):932–937.
18. Staedt J, et al. Dopamine D2 receptor alteration in patients with periodic movements in sleep (nocturnal myoclonus). J Neural Transm Gen Sect. 1993; 93(1):71–74.
19. Garcia-Borreguero D, Larrosa O, Granizo JJ, de la Llave Y, Hening WA. Circadian variation in neuroendocrine response to L-dopa in patients with restless legs syndrome. Sleep 2004; 27(4): 669–673.
20. Okura M, et al. Narcoleptic canines display periodic leg movements during sleep. Psychiatr Clin Neurosci 2001; 55(3):243–244.
21. Schenck CH, Mahowald MW. Motor dyscontrol in narcolepsy: rapid-eye-movement (REM) sleep without atonia and REM sleep behavior disorder. Ann Neurol 1992; 32(1):3–10.
22. Arnulf I, et al. Hallucinations, REM sleep, and Parkinson's disease: a medical hypothesis. Neurology 2000; 25; 55(2):281–288.

18
Circadian and Ultradian Aspects in Narcolepsy

FRANCO FERRILLO and LINO NOBILI
Department of Motor Sciences, Center for Sleep Medicine-DISMR, University of Genova, S. Martino Hospital, Genova, Italy

The excessive daytime sleepiness of narcoleptic patients can be considered as an abnormal distribution of sleep and wakefulness over the twenty-four hours rather than a true hypersomnia. This suggests that the phenotypic features of the narcoleptic syndrome could be explained by a disregulation of the rhythmic sleep organization, resulting from an alteration of the interaction between homeostatic, circadian, circasemidian, and ultradian regulatory processes.
This paper summarizes our experience on the sleep organization of narcoleptic patients in Bed Rest condition and aims at defining the dynamics of NonREM sleep, REM sleep and wakefulness in these patients, by means of a computing simulation of a mathematical model which takes into account the recent knowledge about orexin (hypocretin) deficiency.

I. Circadian Aspects

In 1980 Czeisler and co-workers stated the close relationship between sleep timing and core-body temperature in normal subjects (1). More recently, the circadian distribution of NonREM sleep and its coupling with melatonine rhythms (2) and orexin availability has been confirmed, both in primate models (3) and in humans (4).

In narcoleptic subjects the sleep-wake pattern appears to be disrupted by the intrusion of both sleep during the day and often of wakefulness during the night (5,6,7) leading to the hypothesis of a compensatory rebound of the nocturnal sleep loss into wakefulness. However, a defined, albeit attenuated, circadian pattern, where most sleep is restricted to the night period, persists in ambulatory-monitored narcoleptics (8,9) in the everyday life condition. Broughton *et al.*, (8,10,11) have demonstrated no significant differences in the 24-hour duration of all sleep stages in narcoleptics, in comparison with controls, except for an increase of stage one drowsiness, and no close correlation between night sleep and day sleep duration. The same findings seem to be true in laboratory recordings (12).

II. Circasemidian Aspects

The existence of a daytime period of increased sleep propensity (Nap zone), followed by a prolonged period of wake propensity (Forbidden zone for sleep), leading to a

biphasic daily pattern, referred to as circasemidian (13) or hemicircadian (14), has been suggested (15,16,17) and recently confirmed in temporal isolation (18). The occurrence of this biphasic pattern seems to be independent of the duration of prior wakefulness and to be the expression of a Suprachiasmaticus Nucleus (SCN)-dependent circadian arousal process similar to that existing in primates (19). The amount of daytime sleep is greater and its distribution is different in narcoleptic patients and in controls. The peak of daytime-sleep in patients with narcolepsy occurs about two hours earlier than in normal subjects, under everyday life condition (20) and under conditions of short sleep wake schedule (21) and temporal isolation (22).

III. Ultradian Aspects

The existence of ultradian rhythms in the whole nychtemeron of normal subjects as a continuation of the intra sleep NonREM-REM cycles, has been pointed out by Schulz (23). In normal subjects ultradian oscillations of sleep ability with a periodicity of about ninety minutes, have been observed by means of ultra short schedules (16).

The continuation of a periodicity from night sleep to daytime has been proposed on the grounds of the Basic Rest Activity Cycle postulated by Kleitmann, in 1963 (24). In patients with narcolepsy (21) the ultradian system could be considered as unusually strong and thereby able to interfere with the circadian one (5). Schulz demonstrated the strong ultradian rhythm in narcoleptic patients napping with a clear evidence of continuation of a periodicity from nighttime sleep to daytime sleep (23). Similar findings, together with an intermediate ultradian periodicity of approximately 3.5–4 hours (25,26) have been reported.

IV. Homeostatic Aspects

In normal sleepers, the duration and timing of sleep are known to be significantly correlated to prior wakefulness. As interpreted in the two-process model of sleep regulation (27,28), Slow Wave Sleep should reflect a homeostatic process (process S) that increases in an asymptotically exponential saturation during wakefulness. Its decrease is expressed by the exponential decline of the power density of the EEG delta band (Slow Wave Activity, SWA) in the successive sleep cycles.

Process S interacts with the circadian process and determines sleep timing. The two- process model can be modified to account for the circasemidian sleep propensity and for experimentally-induced reduction of influence of the circadian process (29,30). The hypothesis that narcoleptic patients sleep features could be ascribed to an alteration of the homeostatic sleep regulation has been ruled out (31,32).

V. The Bed-Rest Protocol

In order to highlight the influence of endogenous circadian and circasemidian, ultradian and homeostatic factors, our group studied both normal and narcoleptic patients in an experimental setting of Bed Rest (33). For this purpose a study protocol of 32 consecutive hours of Bed Rest, preceded by 16 hours of daytime sleep deprivation,

was designed. In order to exclude or, at least, attenuate exogenous interfering factors, subjects were recorded in a soundproof isolated room with no social contacts, no information about the time of the day, and exposed to a dim light (10 lux). Subjects were not allowed to get up except for bathroom pauses, to read or listen to the radio; they could ask for soft drinks and meals. Results confirmed the following statements.

No significant differences between narcoleptic patients and controls in sleep duration and in stage distribution were found, except for an increase of awakenings and S1 in narcoleptic patients. In other words, more sleep onsets and sleep offsets were present, sleep being organized in multiple bouts of short duration.

SWA decline was evident in narcoleptic patients during the time span corresponding to the first night period (N1, 23,00–7,00) thus confirming a substantial integrity of the homeostatic process in these patients when stimulated (31,32). However, a robust REM sleep drive was still able to determine sleep onset REM periods (SOREMPs) (31,33).

The time distribution of SWA and REM sleep was studied via frequency analysis and periodograms on SWA time series and REM sleep (33,34) episodes. A circadian distribution with a 24-hour rhythm explaining most of the variance, and a circasemidian one (with a period of about 14 hours) explaining a significant percentage of the variance, were evidenced in the control group. An ultradian periodicity of about 90 minutes was detectable in the control group. Moreover, an around 3-hour periodicity of SWA was detectable, though not significant. In narcoleptic patients two peaks were found in the ultradian frequency span: a major peak centered on a four-hour periodicity, explaining most of the variance, and a secondary one representing the NonREM-REM alternation with a period of 120 minutes. Both in control subjects and in narcoleptic patients, the peaks of SWA and REM episodes recorded during daytime (DT, 7.00–23.00) and during the second night (N2, 23.00–7.00) matched, within an interval of acceptance significantly higher than chance, with the maximum and the minimum of the sinusoidal function, fitting the SWA-REM alternation observed during night N1 (Fig. 1, Fig. 2).

The sleep-wake regulation pattern seems to differ in narcoleptic patients where, with respect to controls, a strong evidence of ultradian rhythm is found. During N1, in narcoleptic patients, either NonREM-REM or REM-NonREM alternation had a period of about 120 minutes, leading to sleep cycles significantly longer than in controls. DT and N2 blocks of sleep usually consisted in a sleep onset REM period followed by a SWA bout and a REM sleep period. During DT and N2, REM sleep maintained the same periodicity as in N1 (120 min), while the period of SWA bouts was roughly doubled (240 min). These results are in accordance with previous observations of Billiard's group (25,26).

Summing up, sleep deprivation of 16 hrs prior to the first experimental night, determines a strong homeostatic pressure in narcoleptic patients, thus allowing a compact sleep pattern during the time span corresponding to N1. During the time span corresponding to DT and to N2, the influence of the homeostatic process can be considered as exhausted. The circadian and circasemidian influences are strongly attenuated or even absent, while there is evidence of a very strong ultradian rhythm (34). Taking into account the weakness of waking state mechanisms and the enhanced properties of REM inducing systems, we can infer that the polyphasic pattern peculiar to the narcoleptic sleep distribution in the Bed Rest condition could be explained by an altered balance and coupling between the circadian and circasemidian drives to sleep

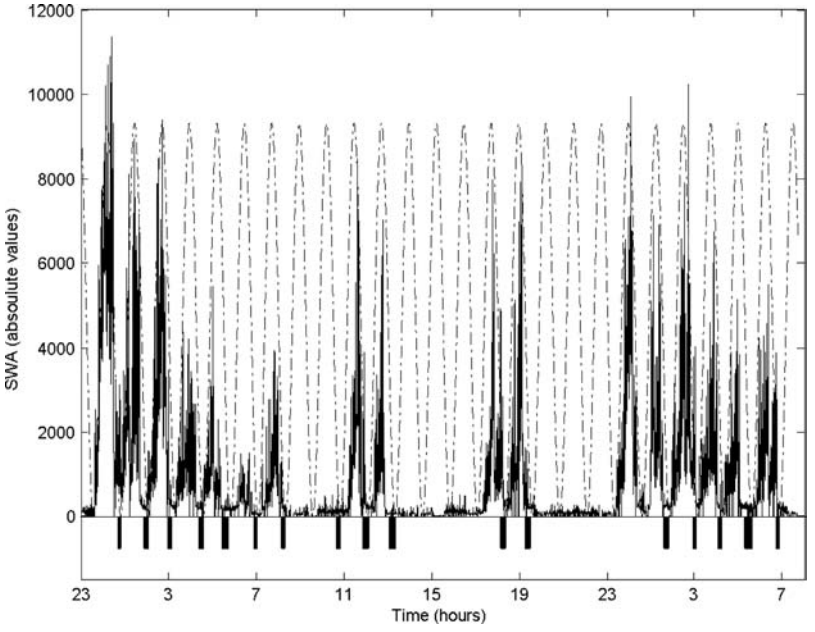


Figure 1 Time course of SWA during 32 hours in a single representative control subject plotted over time with a resolution of 20 seconds. Timing and duration of REM sleep episodes are represented by the vertical black bars. The sinusoid (dashed-dotted line), obtained from the periodicity of SWA in the first night (23.00 −7.00), matches SWA bouts occurring in the successive day and night time with its maxima. REM episodes are synchronous with the minima. Wake episodes are characterized by approaching zero values of SWA in absence of REM.

(attenuated or absent), the homeostatic process (present and efficient if stimulated), and the ultradian drive to REM sleep (strongly enhanced).

The weakness of the circadian and circasemidian drives to sleep in narcoleptic patients could reveal an ultradian drive toward REM sleep, triggering both sleep onset and sleep termination during daytime. The possibility of short wakefulness intervals allows the accumulation of the homeostatic process.

This would result in the polyphasic occurrence of delta pulses discharging the process S accumulated during the preceding episodes of wakefulness. REM sleep could therefore act as a gate mechanism. In turn, this would allow the entering into sleep, the discharge of process S and the shift from sleep into wakefulness where process S accumulation can take place again.

VI. An Attempt to Simulate Features of Sleep in Narcolepsy

The mathematical model we used to simulate the features of sleep consists of a system of four non-linear differential equations, describing the dynamics of homeostatic process, of SWA, and of REM-coupled oscillators. The system has no analytical solution; to solve these kind of differential equations we used the Runge-Kutta numerical method as available in *Matlab* 6.51 software (35).

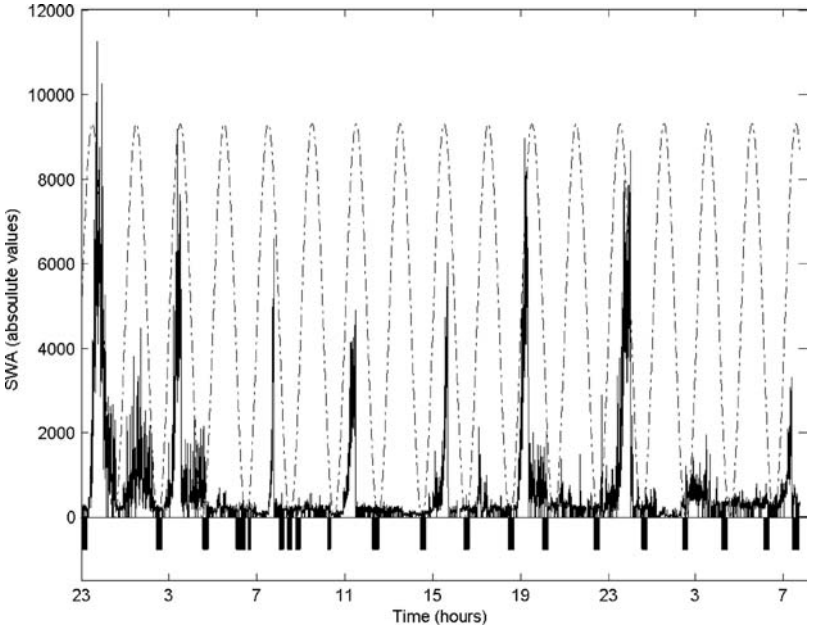


Figure 2 Time course of SWA during 32 hours in a single representative narcoleptic subject plotted over time with a resolution of 20 seconds. Timing and duration of REM sleep episodes is represented by the vertical black bars. The sinusoid (dashed-dotted line), obtained from the periodicity of SWA in the first night (23.00–7.00), matches SWA bouts occurring in the successive day and night time with its maxima. REM episodes are synchronous with the minima. Wake episodes are characterized by approaching zero values of SWA in the absence of REM sleep. SWA bouts and wake episodes, after the first night, show a periodicity of about 4 hours while REM sleep episodes show a periodicity of 2 hours.

The circadian rhythm (process C) is inserted in the model as a permissive condition for both wakefulness and sleep occurrence, according to the extension of the two-process model (28,30). We modeled process C as a simple sinusoidal oscillator with a period of 24 hours. Moreover, a circasemidian peak has been inserted and modelled by a superposition of sinusoidal oscillators with different periods. In the system of non-linear differential equations, the homeostatic process (process S) is characterized by an approximately exponential decrease during NonREM sleep episodes and by an approximately exponential increase during wakefulness and REM sleep episodes (28).

A REM oscillator, characterized by two coupled differential equations (36), has been added on the basis of the reciprocal interaction model suggested by McCarley and Hobson (37). It consists of two coupled, non-linear, differential equations describing the dynamics of RemOn and RemOff variables, being the strength of interactions denoted by parameters of coupling. The interaction between RemOn and RemOff defines the amplitude and the period of pulses allowing REM sleep. The REM pulses are uniformly distributed during the daytime and nighttime, and do not interact directly with process C. The interaction of process S with the high and low threshold of process C and with REM pulses, defines the timing of sleep and wakefulness episodes.

When process S approaches the high threshold, sleep is enabled to begin if and when a REM pulse occurs; when the low threshold of process C is approached by process S, sleep is enabled to finish and wakefulness to begin again if and when a REM pulse comes. The process C thresholds can be exceeded by S until REM pulse occurs.

The ultradian variation of Slow Wave Activity interacting with the REM pulse, defines the timing of REM and NonREM sleep. When SWA values approach and exceed REM pulse values, REM sleep finishes and NonREM sleep or wakefulness episodes are enabled to begin. The point of intersection between SWA and RemOn pulses is meant to be a threshold allowing the occurrence of REM sleep. This condition is valid also for sleep onset, since we fixed sleep onset at the maximum value of RemOn pulse and SWA at its minimum value, allowing a SOREMP in controls and in narcoleptic patients.

In order to simulate sleep in the Bed Rest condition, the gap between the high and low thresholds of process C is reduced, by shifting the high threshold toward lower values.

The REM sleep of narcoleptic patients is modeled by the system of RemOn and RemOff equations featured by an inhibitory coupling parameter greater than in controls. In order to simulate the sleep of narcoleptic patients, the amplitude of the C process oscillation is drastically reduced (by a factor 10) compared with the model of normal sleepers, and the gap between the high and low thresholds is further reduced by shifting the high threshold toward lower values.

Focusing on the night period following sleep deprivation, the model depicts the temporal evolution of SWA whose progressively decreasing trend over cycles matches the raw data with a good approximation.

In detail, in controls (Fig. 3), the periodicity of REM sleep results from the dynamic balance between the level of process S and the strength of RemOn oscillator. This produces the temporal windows for REM sleep occurrence whose length progressively increases in the course of the night as process S declines. It is worth noticing that this model allows a SOREMP, thus linking sleep phase 1 to REM periodicity.

In narcoleptic subjects (Fig. 4), the manipulation of the connectivity co-efficient between RemOn and RemOff cells can reproduce the temporal course of SWA progressive decline via a lower number of longer cycles. As to REM timing and duration, the enhanced strength of RemOn cells accounts for an enhanced probability of SOREMPs, a longer periodicity (120 min) of NonREM-REM cycles and a less progressive increase in REM duration overnight.

The simulation of sleep features in Bed Rest conditions in normal sleepers has been obtained by reducing the high threshold of process C (Fig. 5). This implies that the rising of process S, after the awakening from the first night, reaches the reduced high threshold earlier and in coincidence with the supposed flexus due to the circasemidian rhythm, allowing the manifestation of the mid-afternoon peak of sleep propensity. The link between process C, process S and REM oscillator pulses accounts for the occurrence of SWA and REM episodes, both during daytime and the second night in continuation with the NonREM-REM periodicity observed in the first night. Moreover, the features of the model allow SOREMPs during daytime sleep, as frequently observed in this kind of setting. The time course of SWA, REM sleep and wake distribution over the 32 hours of the Bed Rest protocol applied to narcoleptic subjects is depicted in Figure 6. The manipulations we operated depicted the prevalence of the ultradian

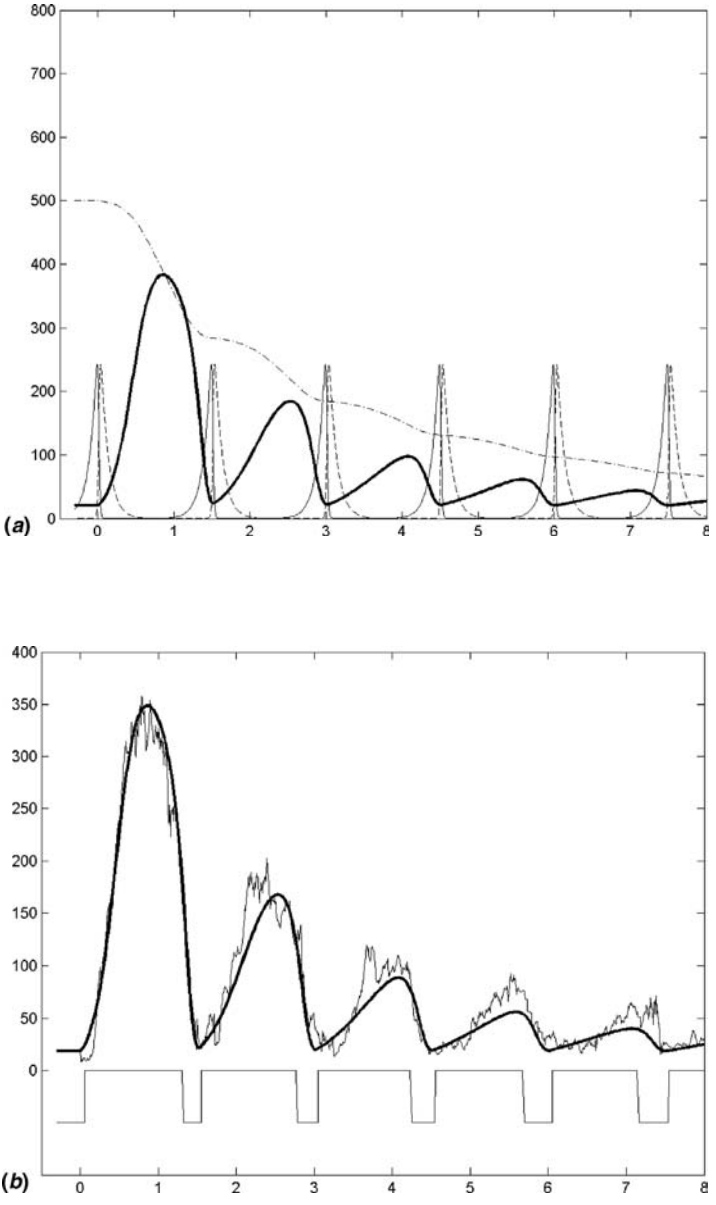


Figure 3 (***a***) Simulation of the ongoing time of the four components of the mathematical model proposed, during the 8 hours of the first night sleep, in the conditions set up for controls (dashed-dotted line, Process S; bold solid line, SWA; thin solid line, RemOn; dashed line, RemOff). (***b***) Comparison between SWA simulation (bold solid line) and time series empirical SWA (thin solid line). Average was obtained from 9 control subjects, by means of moving averaging. Lower graph represents a simulation of the temporal windows for REM sleep obtained according to the model proposed. Both in ***a*** and ***b***, y values are in arbitrary units, and x represents time in hours.

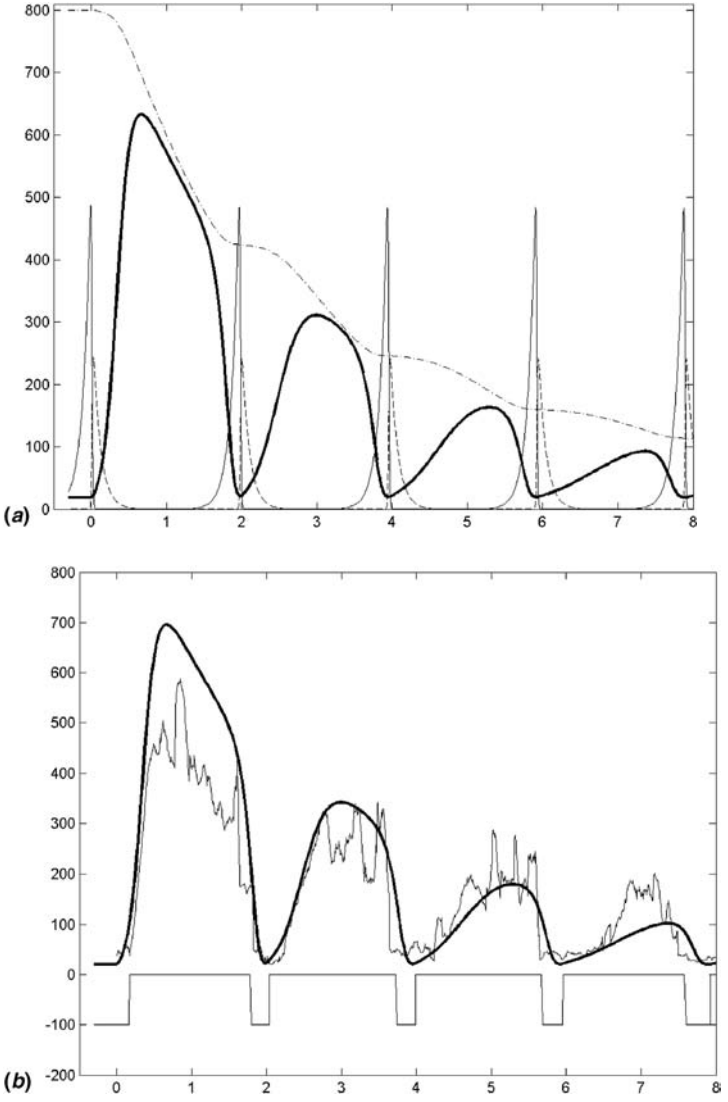


Figure 4 (***a***) Simulation of the ongoing time of the four components of the mathematical model proposed, during the 8 hours of the first night sleep, in the conditions set up for narcoleptic subjects (dashed-dotted line, Process S; bold solid line, SWA; thin solid line, RemOn; dashed line, RemOff). In order to mimic orexin deficiency effects, RemOn-RemOff reciprocal interaction coefficient has been modified producing a stronger RemOn and a longer periodicity of REM pulses compared with controls. (***b***) Comparison between SWA simulation (bold solid line) and time series empirical SWA (thin solid line). Average was obtained from 9 narcoleptic patients by moving averaging. Lower graph represents a simulation of the temporal windows for REM sleep obtained according to the model proposed. Note the greater duration of SOREMP time window and the progressive duration of REM sleep episodes lengthening less pronounced than in controls. Both in ***a*** and ***b***, y values are in arbitrary units, and x represents time in hours.

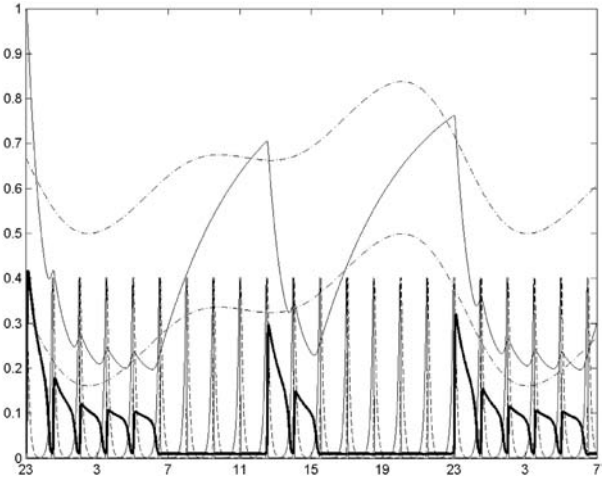


Figure 5 Simulation of ongoing time of the components of the mathematical model proposed over 32 hours in bed rest condition for controls (dashed-dotted line, high and low threshold of process C; thin solid line, process S; bold solid line, SWA; thin solid line, RemOn; dashed line, RemOff, y-values are in arbitrary units, and x represents time in hours). Note that the reduced gap between thresholds of process C allows process S to exceed the threshold, thus permitting a mid-afternoon nap.

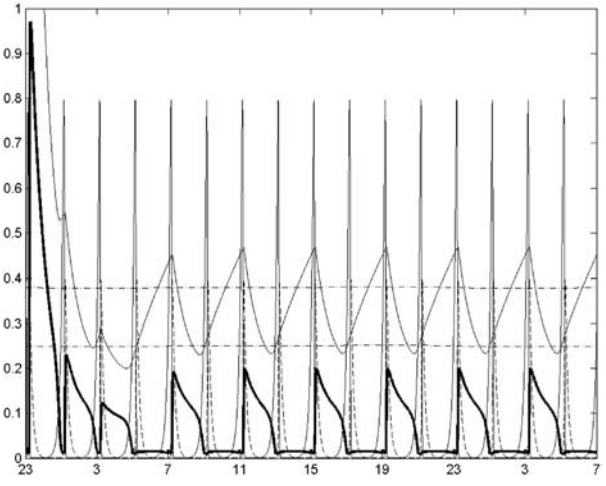


Figure 6 Simulation of ongoing time of components of the mathematical model proposed over 32 hours in bed rest condition for narcoleptic subjects (dashed-dotted line, high and low threshold of process C; thin solid line, process S; bold solid line, SWA; thin solid line, RemOn; dashed line, RemOff, y-values are in arbitrary units, and x represents time in hours). The high and low thresholds of process C are reduced in amplitude and the gap between them is drastically reduced (mimicking orexine deficiency). Conditions imposed to the model produce a peculiar distribution of wakefulness, REM sleep and SWA after the first night. When process S initial strength is exhausted, process S rises till it reaches the high threshold of process C and, in correspondence with a strong REM pulse, it induces a cycle of REM sleep-SWA-REM sleep. When process S values exceed the low threshold, a new wakefulness episode can take place.

distribution of SWA and REM sleep episodes observed in our experimental protocol. The process S decline below the low threshold of process C is achieved after three long REM-SWA cycles. The accumulation of Process S needed to reach the drastically reduced high threshold, is achieved after 120 minutes of wakefulness in coincidence with a REM oscillator pulse. This leads to blocks of REM-SWA-REM at the end of which the declining process S reaches the low threshold of process C. This wakefulness-REM-SWA-REM-wakefulness pattern keeps on reproducing itself during daytime and over the second night.

VII. Comments

The simulation we propose is grounded on models of sleep regulation developed by other authors. Our contribution consists in the proposal of a peculiar interaction modality between the various processes and chiefly on the assumption that REM pulse can play a role in allowing both the sleep cycles onset and offset.

The two-processes model proposed by Borbely (27) is grounded on an interaction between two mechanisms, a homeostatic sleep-dependent one and a circadian one depending on endogenous and exogenous conditions indexed by body temperature and plasma melatonin, and perhaps by orexin (2,3). Although these two processes interact, they operate independently (38). A circasemidian peak in the circadian oscillator was introduced in our model in order to facilitate and to anticipate the timing of the intersection between the rise of process S and the high threshold of process C. The nature of the homeostatic and circadian influences on REM sleep regulation and of the interplay between REM and NonREM sleep are rather complex. It has been proposed (39) that the need for REM sleep increases exclusively during NonREM sleep, thus postulating a somehow subserving function of REM sleep. Other authors postulated a long-term and a short-term homeostatic regulation of REM sleep independent of NonREM sleep (40,41) with an accumulation in the absence of REM sleep during both wakefulness and NonREM sleep (42). However, how REM sleep is regulated and by what, and which is the role of awakenings in the resetting of sleep regulation is still a matter of debate (43,44,45,46,47,48).

The ultradian rhythm model set up by Borbely and his group (28) used empirical REM sleep data to activate the REM sleep trigger parameter. The ultradian process was combined with homeostatic and circadian processes in the extension of the limit cycle reciprocal interaction model. In both these models, the possibility of a sleep onset REM episode is not foreseen and the limit cycle introduces an a priori circadian regulation of REM duration (49,50,51,52). In our model, we assumed an ongoing ultradian process that is maintained during the nychtemeron by an intrinsically sleep-independent generator, whose manifestation is allowed by concomitant conditions of both process S and process C. The process is simulated within the context of the reciprocal interaction model of REM sleep control proposed by McCarley and Hobson (37). This recently reviewed model (53), explains how the REM sleep cycle, both in terms of REM sleep duration and periodicity, may be generated by several pontine nuclei. The model proposes that the neurons of the dorsal raphe and locus coeruleus have an inhibitory collateral autofeedback that eventually stops their own activity and allows the neurons of the laterodorsal tegmental and pedunculopontine nuclei to gain activity

and to generate REM sleep; the strength of these autofeedback connections accounts for intervals between REM episodes. Moreover a regulation of these pontine centers by substances like orexin has been suggested (54). The REM oscillator and its interaction conditions with processes S and C that we propose are sufficient to simulate the wakefulness-REM-NonREM articulation both during the night compact sleep and during daytime nap episodes. This presupposes the attribution to REM sleep of the ability to trigger both the beginning and the end of sleep episodes and of NonREM-REM cycles. The REM pulse would induce periodical brain state instabilities (54) neurophysiologically characterized by EEG drowsiness classifiable as stage1. When and if these instability states are concomitant with levels of process S approaching or exceeding the high threshold of process C, sleep can begin. At this point either REM or NonREM sleep may then occur depending on the balance of their momentary tendencies (47). The duration of REM sleep episodes depends on the competitive interaction between the levels of process S exponentially declining and the cyclic tendency toward REM sleep. Low values of process S concomitant with either the approaching or the exceeding of the low threshold of process C, would lead to wakefulness. This approach could explain the possibility of sporadic SOREMPS at the beginning of a night sleep or of a day sleep cycle and might also serve as an explanation for the so called skipped REM sleep (55,56,57). Similarities in electrophysiological features of drowsiness state (stage 1) and REM sleep have been pointed out by Borbely (27). Moreover, in everyday life, SOREMPs are far from rare while they are rather frequent in some sleep pathologies characterized by an increased sleep pressure (58). It is also suggested that the most common modality of spontaneous awakening is waking up from a REM episode (59). Our simulation is sufficient to account for the lengthening of the duration of REM sleep episodes over the night without imposing homeostatic or circadian dependencies on REM sleep. For this purpose, the competitive interaction between the decaying ongoing of the process S levels and the intensity of REM pulses constant at each peak, is sufficient. The presence of constant REM oscillations during the whole nychtemeron would account also for SOREMPs and ultradian bouts of sleep during daytime with the same periodicity as in the previous night. According to Lavie (16,17) these bouts could be the expression of minor gates to sleep, hidden and overwhelmed by the strength of C and S processes in normal conditions. These hidden gates to sleep in Bed Rest condition could give origin to naps consisting of one or more REM-NonREM cycles occurring at a periodicity of around 3 hours. (17,33). These interpretations are enforced by REM-NonREM sleep-wake patterns recorded from narcoleptic subjects.

Essential information on the pathophysiological basis of narcolepsy has been supplied by genetic and molecular techniques. Experimental studies have shown that (60,61) animals with a loss of orexin or a dysfunction of the lateral hypothalamic orexin system have a phenotype very similar to the human disorder.

Recently a deficiency in orexin has been observed in human narcolepsy (62,63). Orexin is a newly discovered hypothalamic excitatory neuropeptide playing a key role in sleep-wake organization and feeding behaviour (54,64,65,66). It is produced exclusively by a population of neurons in the lateral hypothalamic area, with projections throughout the brain and spinal cord with predominant innervation of monoaminergic and cholinergic centres controlling sleep-wakefulness in the hypothalamus and brainstem (54,60). Experimental studies in primates have shown a circadian distribution of orexin

with low levels during the initial 1-3 hours of wakefulness after sleep, followed by a linear increase and highest level in the latter third of the wake period. The onset of sleep and the concomitant relaxation of the sleep drive cause a decrease in orexin concentrations (3). Orexin is subject both to circadian and homeostatic regulation as the ablation of the suprachiasmatic nucleus abolishes its production, while its levels are enhanced by sleep deprivation (3,67). Therefore, orexin seems to be an essential component of the mechanisms maintaining prolonged wakefulness and opposing to homeostatic sleep propensity (54,68). As for REM sleep it has been found that the tuberomammilary nucleus, the locus coeruleus and the raphe nuclei contain orexin receptors exerting an inhibitory effect on REM sleep (54). Therefore the absence of excitatory orexin input would augment the strength of REM mechanisms, thus facilitating more frequent transitions to REM sleep. Both the reduction of the higher threshold of process C (resulting in a decrease of ability to stay awake) and the increased strength of the RemOn oscillator (resulting in an increase of REM sleep onset) could mimic a deficit of orexin.

Modifying the interconnectivity between RemOn-RemOff cells results in stronger and less frequent REM pulses. The coupling of these modified REM pulses with a normally decreasing process S accounts for a higher possibility of a SOREMP, typical of but not compulsive in narcolepsy. It accounts also for a lower inhibition of REM sleep duration by process S, whose relative importance is reduced. The second manipulation we have done is a drastic reduction of the high threshold of process C. In Bed Rest condition, the reduction of the exogenous *Zeitgebers* is relevant and orexin deficiency prevents the expression of the circadian endogenous influence on sleepiness. The concomitant action of these two factors allows considering circadian modulation as completely irrelevant. During the night period, process S, strongly stimulated by the previous daytime sleep deprivation, allows a rather compact sleep though shorter than in controls.

Two hours of wakefulness fill the interval between the last REM pulse of the night and the next one. During this interval, an accumulation of process S, sufficient to reach the drastically reduced high threshold, takes place. Process S accumulation in such a short interval can generate only one cycle of NonREM sleep terminated by the successive REM pulse. This mechanism continues for a whole day and nighttime giving origin to a cyclic Slow Wave Activity peak every 240 minutes. Hence, the functional unit of the narcoleptic patient spontaneous sleep would be represented by a sequence of REM-NonREM-REM emerging from wakefulness, when REM pulses periodicity would impose onset and offset every 120 minutes and the homeostatic features of process S would impose the rise of a slow wave sleep episode every 240 minutes. This coupling between process S, enforced REM pulses and lowered circadian influences should determine the spontaneous trend of wake NonREM-REM sleep regulation in narcoleptic patients, should they be free to sleep or to wake without taking into account social stimuli and the dark-light cycle.

Though attenuated, this around four-hour ultradian periodicity pattern has been described in controls (17,33). According to Lavie it could be considered as a vestigial trace of a primitive periodicity pattern. A wake-sleep periodicity around four hours is actually detectable in newborns, when awakenings are scheduled by hunger. Since both functions, feeding and waking, are regulated by orexin, data on the bioavailability of this peptide during ontogenesis would be most welcome and could favor reflections on the successive development and divaricating between the two functions.

References

1. Czeisler CA, Weitzman E, Moore-Ede MC, Zimmerman JC, Knauer RS. Human sleep: its duration and organization depend on its circadian phase. Science 1980; 4475:1264–1267.
2. Tagaya H, Uchiyama M, Shibui K, Kim K, Suzuki H, Kamei Y, Okawa M. Non-rapid-eye-movement sleep propensity after sleep deprivation in human subjects. Neurosci Lett. 2002; 323:17–20.
3. Zeitzer JM, Buckmaster CL, Parker KJ, Hauck CM, Lyons DM, Mignot E. Circadian and Homeostatic Regulation of Hypocretin in a Primate Model: Implications for the Consolidation of Wakefulness. J Neurosci 2003; 23:3555–3560.
4. Salomon RM, Ripley B, Kennedy JS, Johnson B, Zeitzer JM, Nishino S, Mignot E. Diurnal variation of CSF hypocretin-1 (orexin-A) levels in control and depressed subjects. Biol Psychiatry 2003; 54: 96–104.
5. Kripke D. Biological rhythm disturbances can cause narcolepsy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy. Spectrum: New York, 1976:475–483.
6. Pavel S, Goldstein R, Petrescu M. Vasotocin melatonin and narcolepsy: possible involvement of the pineal gland and its pathophysiological mechanism. Peptides 1981; 2:245–250.
7. Mosko SS, Holowach JB, Sassin JF. The 24-hour rhythm of core temperature in narcolepsy. Sleep 1983; 137–146.
8. Broughton R, Dunham W, Newman J, Lutley K, Duschesne P, Rivers M. Ambulatory 24 hour monitoring in narcolepsy-cataplexy compared to matched controls. Electroencephalogr Clin Neurophysiol 1988; 70: 473–81.
9. Dantz B, Edgar DM, Dement WC. Circadian rhythms in narcolepsy: Studies on a 90-minute day. Electroencephal Clin Neurophysiol 1994; 90:24–35.
10. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired Circadian Waking Arousal in Narcolepsy-Cataplexy. Sleep Research Online 1998; 1:159–165.
11. Broughton R. Some underemphasized aspects of sleep onset. In: Ogilvie R, Harsh J, eds Sleep Onset Mechanism. Arlington Va: American Psychological Association 1994; 19–36.
12. Harsh J, Peszka J, Hartwig G, Mitler M. Night-time sleep and daytime sleepiness in narcolepsy. J. Sleep Res 2000; 9: 309–316.
13. Broughton R. Biorhythmic variations in consciousness and psychological functions. Can Psychol. Rev 1975; 16: 217–239.
14. Kronauer RE, Jewett ME.The relationship between circadian and hemicircadian components of human endogenous temperature rhythms. J Sleep Res 1992; 2:88–92.
15. Czeisler C, Allen JS, Strogatz SH. Bright light resets the human circadian pace maker independent of the timing of the sleep wake cycle. Science 1986; 233: 666–671.
16. Lavie P, Scherson A. Ultrashort sleep - waking schedule. 1. Evidence of ultradian rhythmicity in 'sleepability'. Electroen Clin Neuro 1981;52:163–174.
17. Lavie P. Ultrashort sleep - waking schedule. 3. 'Gates' and 'forbidden zones' for sleep. Electroen Clin Neuro 1986; 63:414–425.
18. Hayashi M, Morikawa T, Hori T. Circasemidian 12 h cycle of slow wave sleep under constant darkness. Clin Neurophysiol 2002; 113: 1505–1516.
19. Edgar DM, Dement WC, Fuller CA. The effect of SCN lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. J Neurosci 1993; 13:1065–1079.
20. Mullington J, Newman J, Dunham W, Broughton R. Phase timing and duration of naps in narcolepsy-cataplexy: preliminary findings. In: Horne J, ed. Sleep '90, Bochum UK: Pontenagel, 1990:158–160.
21. Lavie P. REM periodicity under ultrashort sleep/wake cycle in narcoleptic patients. Can J Psychol 1991;45:185–193.
22. Pollak CP, Wagner DR, Moline ML, Monk TH. Cognitive and motor performance of narcoleptic and normal subjects living in temporal isolation. Sleep 1992; 15: 202–211.
23. Schulz H. Ultradian rhythms in the nycthemeron of narcoleptic patients and normal subjects.In: Schulz H, Lavie P: Ultradian Rhythms in Physiology and Behaviour. New York - Berlin, Springer Verlag 1985: 165–185.
24. Kleitman N. Sleep and Wakefulness. 2d ed. Chicago: Chicago University Press, 1963.
25. De Koninck J, Salva MQ, Besset A, Billiard M. Are REM cycles in narcoleptic patients governed by an ultradian rhythm?. Sleep 1986; 9:162–166.

26. Billiard M, De Koninck J, Coulombe D, Touzery A. Napping behaviour in narcoleptic patients: a four hours cycle in slow wave sleep. In: Stampi C. Why we nap: Evolution, Chronobiology and Functions of Polyphasic and Ultrashort Sleep. eds. Birkauser, Boston, MA 1992:245–257.
27. Borbely AA. A two-process model of sleep regulation. Hum Neurobiol 1982; 1:195–204.
28. Borbély AA, Achermann, P. Concepts and models of sleep regulation: an overview. J Sleep Res 1992; 1: 63–79.
29. Borbely AA, Achermann P. Homeostasis and models of sleep regulation.J Biol Rhythms 1999; 14:557–568.
30. Daan S, Beersma DGM, Borbely AA. Timing of human sleep: recovery process gated by a circadian pacemaker. Am J Physiol 1984; 246:161–183.
31. Tafti M, Rondouin G, Besset A, Billiard M. Sleep deprivation in narcoleptic subjects: effect on sleep stages and EEG power density. Electroencephalogr Clin Neurophysiol 1992; 83:339–349.
32. Besset A, Tafti M, Nobili L, Billiard M. Homeostasis and narcolepsy. Sleep 1994; 17: 29–34.
33. Nobili L, Besset A, Ferrillo F, Rosadini G, Schiavi G, Billiard M. Dynamics of slow wave activity in narcoleptic patients under bed rest conditions. Electroen Clin Neuro 1995; 95:414–425.
34. Nobili L, Ferrillo F, Besset A, Rosadini G, Schiavi G, Billiard M. Ultradian aspects of sleep in narcolepsy. Neurophysiol Clin 1996; 26:51–59.
35. MATLAB 6.51, © 1994–2004 The MathWorks, Inc.
36. Nobili L, Beelke M, Besset A, Billiard M, Ferrillo F. Noctural sleep features in narcolepsy: a model-based approach. Rev Neurol 2001; 157: 82–86.
37. McCarley RW, Hobson JA. Neuronal Excitability modulation over the sleep cycle: A structural and mathematical model. Science 1975; 189:58–60.
38. Trachsel L, Edgar DM, Seidel WF, Heller HC, Dement W. Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. Brain Res 1992; 589:253–261.
39. Benington J. H, Heller HC. REMS timing is controlled homeostatically by accumulation of REMS propensity in non-REMS. Am J Physiol 1994; 266:1992–2000.
40. Ocampo Garces A, Molina E, Rodriguez A, Vivaldi EA. Homeostasis of REMS after total and selective sleep deprivation in the rat. J Neurophysiol 2000; 84:2699–2702.
41. Ocampo Garces A, Vivaldi EA. Short-term homeostasis of REM sleep assessed in an intermittent REM sleep deprivation protocol in the rat. J Sleep Res 2002; 11:81.
42. Franken P. Long-term vs. short-term processes regulating REM sleep. J Sleep Res 2002; 11:17–28.
43. Villablanca JR, De Andrés I, Garzón M. Debating how rapid eye movement sleep is regulated (and by what). J Sleep Res 2003, 12:259–262.
44. Benington JH. Debating how REM sleep is regulated (and by what). J Sleep Res 2002; 11:29–31.
45. Franken P, Response. Debating how REM sleep is regulated (and by what). J Sleep Res 2002; 11:31–33.
46. Rechtschaffen A, Bergman BM. Sleep rebounds and their implications for sleep stages substrates: a response to Benington and Heller. Sleep 1999; 22:1038–1043.
47. Grozinger M, Beersma DG, Fell J, Roschke J. Is the nonREM–REM sleep cycle reset by forced awakenings from REM sleep?. Physiol Behav 2002; 77:341–347.
48. Barbato G, Barker C, Bender C, Wehr TA. Spontaneous sleep interruptions during extended nights. Relationships with NREM and REM sleep phases and effects on REM sleep regulation. Clin Neurophysiol 2002; 113:892–900.
49. McCarley RW, Massaquoi SG. Neurobioogical structure of the revisited Limit Cycle Reciprocal Interaction Model of REM cycle control. J Sleep Res 1992; 1:132–138.
50. Massaquoi SG, McCarley RW. Extension of the limit cycle reciprocal interaction model of REM cycle control. An integrated sleep control model. J Sleep Res 1992; 1:138–143.
51. Achermann P, Borbely AA. Combining different models of sleep regulation. J Sleep Res 1992; 1:144–147.
52. Achermann P, Beersma DG, Borbely AA. The two-process model: ultradian dynamics of sleep. In: Horne JA, eds Sleep '90 Pontenagel Press, Bochum, 1990:310–314.
53. Pace-Schott EF, Hobson JA. The neurobiology of sleep: genetics, cellular physiology and subcortical networks. Nat Rev Neurosci 2002; 8:591–605.
54. Saper CB, Chou TC, Scammel TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24:726–731.

55. Nicholson AN, Belyavin AJ, Pascoe PA. Modulation of rapid eye movement sleep in humans by drugs that modify monoaminergic and purinergic transmission. Neuropsychopharmacology 1989; 2:131–143.
56. Belyavin A, Nicholson AN. Rapid eye movement sleep in man: modulation by benzodiazepines. Neuropharmacology 1987; 26:485–491.
57. Feinberg I, March JD. Cyclic delta peaks during sleep: result of a pulsatile endocrine process?. Arch Gen Psychiatry 1988; 45:1141–114 2.58.
58. Chervin RD, Aldrich MS. Sleep Onset REM periods during Multiple Sleep Latency Tests in Patients Evaluated for Sleep Apnea. Am J Respir Crit Care Med 2000; 161:426–431.
59. Akerstedt T, Billiard M, Bonnet M, Ficca G, Garma L, Mariotti M, Salzarulo P, Schulz H. Awakening form sleep. Sleep Med Rev 2002; 6:267–286.
60. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammel T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Ficth TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–445.
61. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, De Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–376.
62. Nishino S, Ripley B, Overern S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
63. Peyron C, Franco J, Rogers W, Ripley B, Overeen S, Chamay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouracs C, Kucherlapati R. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
64. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozloski GP, Wilson S, Arch JSR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ. Orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.
65. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia and obesity. Neuron 2001; 30:345–354.
66. Taheri S, Zeitzer JM, Mignot E. The role of hypocretins (orexins) in sleep regulation and narcolepsy. Annu Rev Neurosci 2002; 25:283–313.
67. Zhang S, Zeitzer JM, Yasushi Y, Wisor JP, Nishino S, Edgar DM, Mignot E. Lesions of the Suprachiasmatic Nucleus Eliminate the Daily Rhythm of Hypocretin-1 Release. Sleep 2004; 27: 619–627.
68. Mignot E, Taheri S, Nishino S. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nature Neurosci 2002; 5:1071–1075.

19
Homeostatic Sleep Regulation in Narcolepsy

RAMIN KHATAMI
Department of Neurology, University Hospital, Zurich, Switzerland

PETER ACHERMANN and HANS-PETER LANDOLT
Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland

CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

I. Introduction

Sleep homeostasis refers to a regulatory process that counteracts transitory deviations of sleep from an average "reference level" (1). In the two-process model, a homeostatic (process S) and a circadian process (C) determine timing, duration and structure of sleep and wakefulness (2). Homeostatic process S and circadian process C interact to allow consolidated periods of sleep and wakefulness: the gradual increase of process S during waking is counterbalanced by a declining trend of endogenous sleep propensity, and the inverse relationship exists during sleep (Fig. 1).

Both states, waking and sleep, are instable in narcolepsy-cataplexy. Chronic sleepiness and sleep attacks occur during the daytime, while sleep at night is fragmented. Sleep periods often start with REM-sleep (sleep onset REM-sleep; SOREMs). The inability to consolidate a behavioral state is also evidenced by the occurrence of dissociated features such as cataplexy, in which REM sleep atonia occurs during wakefulness or sleep paralysis, in which atonia occurs at sleep onset. It has been hypothesised that the inability to consolidate either sleep or wakefulness is a consequence of abnormal sleep pressure caused by abnormal homeostatic regulation (4–8). The process reflecting sleep homeostasis can be derived from the time course of EEG slow wave activity (SWA; power within 0.75–4.5 Hz) in NREM-sleep. NREM sleep intensity as indexed by SWA changes as a function of prior wakefulness: SWA is enhanced after total sleep deprivation and is reduced after daytime sleep (1). Recently it has been shown, that theta activity (5–8 Hz) in the wake EEG reflects NREM-sleep homeostasis during wakefulness (3). Contrary to NREM-sleep, no EEG-markers have been identified to reflect the intensity of REM-sleep. The homeostatic regulation of REM-sleep can be derived from (*i*) the increasing number of interventions needed to prevent REM-sleep within a single night and across consecutive nights and (*ii*) the rebound of REM-sleep induced by selective REM-sleep deprivation. REM-sleep rebound is minimal in the night immediately after REM-sleep deprivation. In

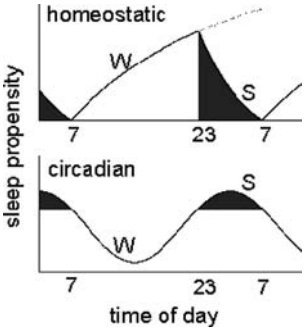


Figure 1 Two-process model of sleep regulation. Schematic representation of the two major processes underlying sleep regulation. *Abbreviations*: W, waking; S, sleep.

summary, total sleep deprivation and selective REM-sleep deprivation are useful tools to investigate NREM- and REM-sleep homeostasis. Only a few studies applied sleep-manipulating experiments to investigate NREM- and REM-sleep homeostasis and its effect on daytime sleepiness and cataplexy in narcoleptic patients (4–8).

II. NREM-Sleep Homeostasis in Human Narcolepsy

Recordings of daytime sleep of narcoleptic patients under two different regimens demonstrated that the amount of SWS during the day influenced the amount of SWS in the subsequent night. Patients who were required to stay in bed during the day ("bed group") had a higher amount of SWS during spontaneous daytime sleep compared to a "table group" who napped in a sitting position. During the subsequent night, SWS was higher in the "table group" than in the "bed group" suggesting that homeostatic NREM-sleep regulation is functional in narcoleptic patients (9). This study, however, did not include a control group, and SWA as a physiological indicator of sleep intensity was not measured. The effect of total sleep deprivation on sleep stages and SWA in NREM-sleep was investigated in other studies (4,5). Following a 24-hour waking period, SWA was increased compared to baseline values in normal subjects and narcoleptic patients, and the relative increase was even higher in the narcoleptic patients. SWA enhancement was most prominent in the first sleep cycle and attenuated in the second and third cycle. SWA dissipated exponentially across NREM-sleep with a similar time course in both groups. The authors concluded that homeostatic NREM-sleep regulation is intact, but sleep deprivation induced a stronger response in narcoleptic patients (4). A study comparing the time course of SWA in NREM-sleep after daytime sleep deprivation (i.e., 16-hour waking period during the daytime) and during a 32 hour bed-rest condition confirmed the notion of an intact, but quantitatively different homeostatic regulation in NC-patients. SWA exponentially declined after diurnal sleep-deprivation in NC-patients and in normal controls (8). During the second night of the 32 hour bed-rest condition only normal controls showed an exponential decline of SWA (although starting from a reduced level). In contrast, NC-patients had no decay of SWA, consistent with a prominent attenuation of NREM sleep pressure (due to abundant daytime sleep).

In these studies homeostatic regulation was estimated indirectly on the basis of SWA in baseline and recovery nights, providing limited insight into NREM-sleep homeostatic regulation during wakefulness. As process S is a direct function of prior wakefulness it would be instructive to measure the evolution of sleep homeostasis during waking. The homeostatic build-up of NREM-sleep propensity (as determined by theta-activity in the wake EEG) during a sustained waking time was compared in HLA DQB1*0602 positive, drug free narcolepsy with cataplexy patients and age and sex-matched controls (Khatami, unpublished results, in preparation). Sleep deprivation for 40 hours started the morning after a baseline night and ended in the evening of the next day. Consequently, recovery sleep was scheduled to begin at the same circadian time as baseline sleep. During the sleep deprivation period, participants stayed in the laboratory under continuous face-to-face observation to avoid even short naps. Wake EEGs were recorded in 14 sessions at 3-hour intervals. The preliminary results of five patients with narcolepsy and four controls suggest a different evolution of theta activity (EEG power within 5–8 Hz) during the sleep deprivation period. Theta activity increased in the healthy controls during prolonged wakefulness and a circadian modulation was evident. By contrast, the narcoleptic patients showed a declining trend of theta activity during the first 27-hour of sleep deprivation and an increase afterwards. Subjective sleepiness (Stanford Sleepiness Scale) increased across 40-hour in both groups and did not differ between NC-patients and healthy controls. There was no increase in the number of cataplexies in NC-patients during the entire waking period. Consistent with prior reports, sleep deprivation enhanced SWA in both NC and healthy controls. In our study, however, the rise of SWA was not different between the two groups. Remarkably, even after a prolonged waking period of 40 hours all patients started recovery sleep with REM-sleep (SOREMs). This finding is in accordance with the previous observation that SOREMs in narcoleptic patients occurred after 16 and 24 hours wakefulness (4,5). Contrary to these studies, however, the SOREMs after 40 hours waking time cannot be attributed to a possible circadian effect as recovery sleep started at a time of low circadian REM-sleep pressure.

III. REM-Sleep Homeostasis in Human Narcolepsy

Selective REM-sleep deprivation for one night resulted in a number of interventions needed to prevent REM sleep that was twice as high in patients with narcolepsy as in normal controls (6). Numbers of interventions were not equally distributed throughout the night, but peaked at the beginning (due to SOREMs) and at the end of the night. Cumulative data across the night indicated a stronger increase of interventions in narcoleptic patients in the last third of the night. On MSLT performed the day after selective REM-sleep deprivation, patients had a significantly higher number of SOREMs relative to a baseline MSLT performed one week before. In normal subjects, the number of REM-sleep interventions correlated with a short sleep latency on baseline MSLT, whereas in narcoleptic patients an inverse correlation was noted. In other words, normal subjects (but not patients with narcolepsy) who were sleepier on a baseline MSLT appeared to have a higher REM-sleep pressure during nocturnal sleep. No data were reported on sleep latencies or REM-sleep latencies of MSLT following the

deprivation night. In a recent study, NC-patients were deprived of REM-sleep for two consecutive nights followed by MSLT and a recovery night (7). Consistent with the prior study, the number of interventions in the first deprivation night was nearly twice as high in NC-patients as in normal controls. In the second deprivation night, an additional increase of interventions was found in both groups (53% in NC-patients and 46% in the healthy controls). The interventions per 2-hour time intervals increased across the night in a nonlinear fashion and were similar in both nights. REM-sleep deprivation induced subjective daytime sleepiness (Stanford Sleepiness Scale and visual analog scales), which was pronounced in NC-patients and mild in healthy controls. The number of cataplectic attacks did not change after REM-sleep deprivation. On MLST, only NC-patients had an increase of SOREMs and a non-significant decrease in sleep latency and REM-sleep latency. During the recovery night, no changes either in sleep stages, total sleep time or NREM- and REM-sleep latencies were found in either group when compared to the corresponding baseline values.

IV. Discussion

Available data suggest qualitatively intact NREM- and REM-sleep homeostasis in narcolepsy, whereas quantitative differences may exist when compared to normal subjects. The qualitative functioning of NREM-sleep homeostasis is evidenced by an increase in SWA in recovery sleep following sustained wakefulness and its exponential decline during the recovery night. Patients with narcolepsy may be more sensitive to increased sleep pressure (4) as homeostatic response to sleep deprivation was enhanced. Our preliminary data indicate a different temporal evolution of theta activity during wakefulness, which could be related to a disturbed interaction of the homeostatic and the circadian systems in narcolepsy. Remarkably, increased NREM-sleep pressure following 40-hour wakefulness did not prevent SOREMs in recovery sleep. Increased REM-sleep pressure was reflected in a higher amount of SOREMs during both daytime sleep (on MLST) and increased numbers of interventions to prevent REM-sleep during nighttime. Again, homeostatic REM-sleep regulation appears to be intact, as the increasing number of interventions across consecutive REM-sleep deprivation nights was similar in NC-patients and healthy controls. A rapid decrease of homeostatic NREM-sleep drive (4) may promote an additional disinhibition of REM-sleep towards the end of the night. Together with a circadian peak of REM-sleep propensity, this would explain the high number of interventions needed to prevent REM-sleep in the last third of the night. REM-sleep pressure remained high in daytime (number of SOREMs increased), but no REM-sleep rebound occurred during the recovery night. Daytime sleepiness or catapletic attacks (7) are not consistently related to the increase of either NREM- (5) or REM-sleep pressure (7).

V. Conclusions

Homeostatic NREM- and REM-sleep regulation are functional in narcolepsy, yet may operate at a different level compared with healthy people. It remains difficult to quantify the net effect of altered homeostatic NREM-/REM-sleep pressure and their

interaction with the wake-promoting system. At present there is no consistent evidence that excessive daytime sleepiness and REM sleep associated features (cataplectic attacks) are affected or even caused by abnormal sleep homeostatic processes. It is more plausible that the loss of a hypothetical "neuronal glue" facilitates multiple transitions between all behavioral states as originally proposed by Broughton (10). Broughton's model of "state boundary dyscontrol" was recently supported by the discovery of hypocretin/orexin deficiency in human and animal narcolepsy. Nevertheless further studies are needed to establish whether hypocretins/orexins represent this "neuronal glue" that integrates and consolidates the behavioral states.

References

1. Borbély AA, Achermann P. Sleep homeostasis and models of sleep regulation. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. Philadelphia: Elsevier Saunders, 2005: 405–417.
2. Borbély AA. A two-process model of sleep regulation. Hum Neurobiol 1982; 1:195–204.
3. Finelli LA, Baumann H, Borbèly AA, Achermann P. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 2000; 101:523–529.
4. Tafti M, Rondouin G, Besset A, Billiard M. Sleep deprivation in narcoleptic subjects: effect of sleep stages and EEG power density. Electroencephal Clin Neurophysiol 1992; 83:339–349.
5. Tafti M, Villemin E, Carlander B, Besset A, Billiard M. Sleep in human narcolepsy revisited with special reference to prior wakefulness duration. Sleep 1992; 15:344–351.
6. Spielman AJ, Adler JM, Glovinsky PB, Pressman MR, Trophy MJ, Ellman SJ, Ackerman KD. Dynamics of REM sleep in narcolepsy. Sleep 1986; 9:175–182.
7. Vu H, Roth C, Mathis J, Hurni C, Achermann P, Bassetti C. REM sleep deprivation in narcoleptics. Sleep Research Online 1999; 2(suppl 1):463.
8. Nobili L, Besset A, Ferrillo F, Rosadini G, Schiavi G, Billiard M. Dynamics of slow wave activity in narcoleptic patients under bed rest conditions. Electroencephalogr Clin Neurophysiol 1995; 95: 414–425.
9. Volk S, Schulz H, Yassouridis A, Wilde-Frenz J, Simon O. The influence of two behavioural regimes on the distribution of sleep and wakefulness in narcoleptic patients. Sleep 1990; 13:136–142.
10. Broughton R, Valley V, Aguirre M, Roberts J, Suwalski W, Dunham W. Excessive daytime sleepiness and the pathophysiology of narcolepsy-cataplexy: a laboratory perspective. Sleep 1986; 9:05–215.

20
Daytime Variations in Alertness/Drowsiness and Vigilance in Narcolepsy/Cataplexy

ROGER J. BROUGHTON
Ottawa Health Research Institute, Ottawa Hospital and University of Ottawa, Ottawa, Canada

This paper concerns four aspects of the daily variations encountered both in daytime alertness/drowsiness levels and in so-called psychomotor vigilance in narcolepsy-cataplexy. They are: (*i*) the physiological changes used as measures of alertness/drowsiness; (*ii*) the issue of whether or not qualitatively different forms of sleepiness and drowsiness exist; (*iii*) the effects of the variations in alertness/drowsiness upon sensitive performance tasks, in particular on so-called vigilance tests; and (*iv*) the underlying mechanism(s) of drowsiness in narcolepsy. The word drowsiness is generally employed rather than sleepiness, as it denotes an objective physiological state, whereas sleepiness has a much broader and less precise meaning.

Although the related literature on narcolepsy generally emphasizes the subjective and the objective physiological aspects of drowsiness in narcolepsy/cataplexy, it is essential to keep in mind that there are objective behavioral changes as well. The transition along the continuum from wakefulness to sleep may progressively include a blank stare, eyelid drooping, slumping of the head and later of the upper body, and then general body slumping along with slow deep regular respirations. SOREMPs in narcolepsy (and in other conditions) may be evident by jerking movements of the eyeballs and eyelids, facial twitching, peripheral twitching of fingers and toes, marked evident background hypotonia of skeletal muscles, irregular respiration and at times moaning.

I. Physiological Measures of Daytime Alertness/Drowsiness Variations in Narcolepsy

The earliest physiological descriptions of the overall marked increase in drowsiness exhibited by persons with narcolepsy-cataplexy came from diagnostic EEG studies. Daly and Yoss in 1957 remarked: "Persistent drowsiness characterizes these patients. This occurs early in the course of the examination but only rarely passes into the stages of light sleep" (1). Similarly, Hishikawa and Kaneko noted in 1965: "A persistent and intense inclination to fall into a drowsy state or sleep characterizes the basic disturbance of narcoleptics" (2).

These and many other early reports based upon routine diagnostic EEGs in patients with narcolepsy-cataplexy emphasized the high proportion of recording time showing patterns of drowsiness or light sleep. As well, such studies defined a number of further common characteristics of narcolepsy. These include: (*i*) the so-called "paradoxical blocking" of the alpha rhythm in which a stimulus during drowsiness induces a burst of alpha rhythm rather than blocking it, as occurs in full wakefulness; (*ii*) the persistence of physiological drowsiness even during the sustained effort involved in the standard 3-minute testing of the effects of hyperventilation and during the intense cerebral activation induced by intermittent photic stimulation; and, (*iii*) the frequent denial of evident prior drowsiness by patients immediately upon return to full EEG and behavioral wakefulness (1–3).

The evolution of the spectrum of EEG changes encountered between full wakefulness and stage 2 sleep can be quite subtle. Although the phrase "stage 1 *sleep*" is frequently used, this state is in fact one of drowsiness rather than of true sleep, as both the subjective and objective features of sleep are typically only present once stage 2 or REM sleep patterns have appeared (4).

Gastaut and Broughton (5) differentiated two sub-stages, 1A and 1B, between wakefulness and stage 2 sleep with the former (1A) being characterized by slowing (by 1 Hz or more) and/or anterior diffusion and fragmentation of the alpha rhythm, with or without slow eye movements; and the latter (1B) consisting of the medium voltage, mixed frequency mainly theta activities that characterize stage 1 of Rechtschaffen, Kales and collaborators (6). A similar breakdown of this waxing and waning contiuum was the early one of Loomis et al. (7) whose stages A and B are essentially identical to sub-stages 1A and 1B, after which in fact they were named. As late as 1962 some workers such as Oswald, in his remarkable volume (8), continued to use the same nomenclature and criteria as Loomis et al. Such distinctions are crucial, as even the minor shift from EEG patterns of full wakefulness to those of substage 1A (minor drowsiness) has a profound effect on performance capacity, as will be discussed later.

Other authors have proposed many more subdivisions. Hori et al. described a half dozen or more sub-stages based on EEG criteria going from full wakefulness to stage 2 sleep (9). Simon et al. (10), employing polygraphic criteria that included the level of muscle tone and the amount of movement artifact (i.e., preamplifier blocking), distinguished 6 sub-state levels within wakefulness alone. This group of Schulz and his collaborators (11) has shown that seated narcoleptics, compared to seated controls, show greater amounts of active wakefulness (i.e., with artifacts) and lower amounts of quiet wakefulness (i.e., waking periods without artifacts) while performing challenging psychomotor tasks.

These findings support the belief that persons with narcolepsy (and perhaps sleepy people in general), at least when making a concentrated effort to perform at high levels of efficiency, tend to move more, or at least tense their muscles more, in order to fight off drowsiness and remain awake. Incompletely published actigraph studies from my laboratory have shown overall lesser amounts of movement in untreated narcolepsy when patients are not involved in performance or similar tasks. This appears to be attributable to the increased amount of drowsiness accompanying relative inactivity.

The duration of periods of daytime drowsiness in patients with narcolepsy varies across a wide spectrum. So-called "microsleeps" lasting some 3 to 15 seconds, and

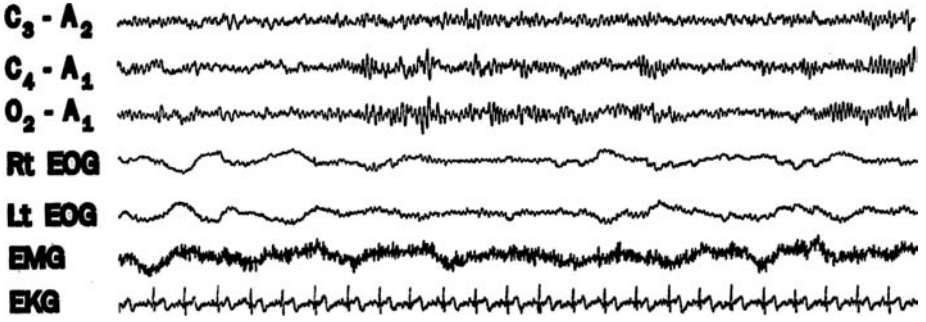


Figure 1 Waxing and waning of EEG patterns in a patient with narcolepsy-cataplexy recorded by ambulatory monitoring (Medilog 9000, Oxford Medical Systems, Abingdon, UK) which shift rapidly from stage 1B, to stage 1A and back, associated with slow eye movements throughout this short sample taken from several minutes of such oscillating patterns.

usually consisting of stage 1B patterns, are well documented. These were first identified using physiological monitoring by Guilleminault and collaborators (12) who described brief EEG shifts from wakefulness into stage 1 with slow nystagmoid eye movements in the electro-oculogram (EOG) channels, the new patterns being associated with temporary cessation of performing a continuous alternation task. Some authors have proposed that the sensitivity and specificity of the MSLT to detect excessive daytime sleepiness can be increased by combining the number of microsleep episodes outside of the scheduled naps with the sleep latencies of the naps themselves (13).

A more durable "waxing and waning" of EEG patterns of alertness/drowsiness in which the fluctuations occur over periods of dozens of seconds or several minutes is often prominent in prolonged daytime recordings of patients with narcolepsy while off stimulant medication. Examples from ambulatory polysomnography are shown in Figures 1 and 2 and in a characteristic histogram in Figure 3. One might expect that a sustained intense effort to attend to or respond to stimuli, or to perform at peak, is more likely to be interrupted by recurrent brief "microsleep" episodes, whereas

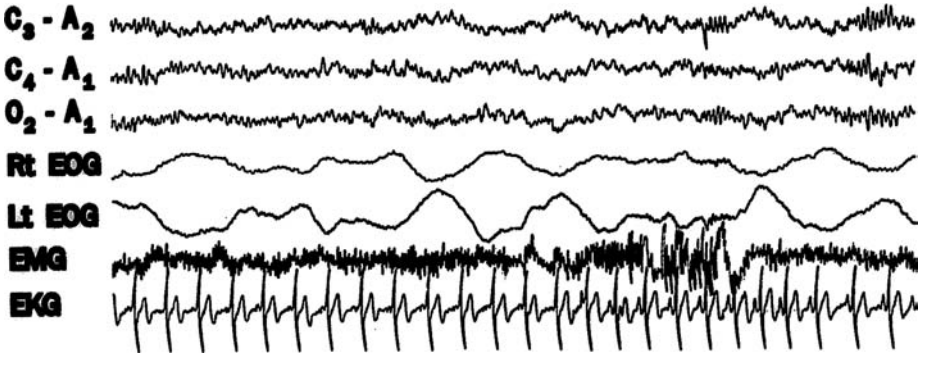


Figure 2 Similar patterns in another patient similarly recorded but with greater theta and delta activity of low amplitude associated with larger amplitude slow eye movements.

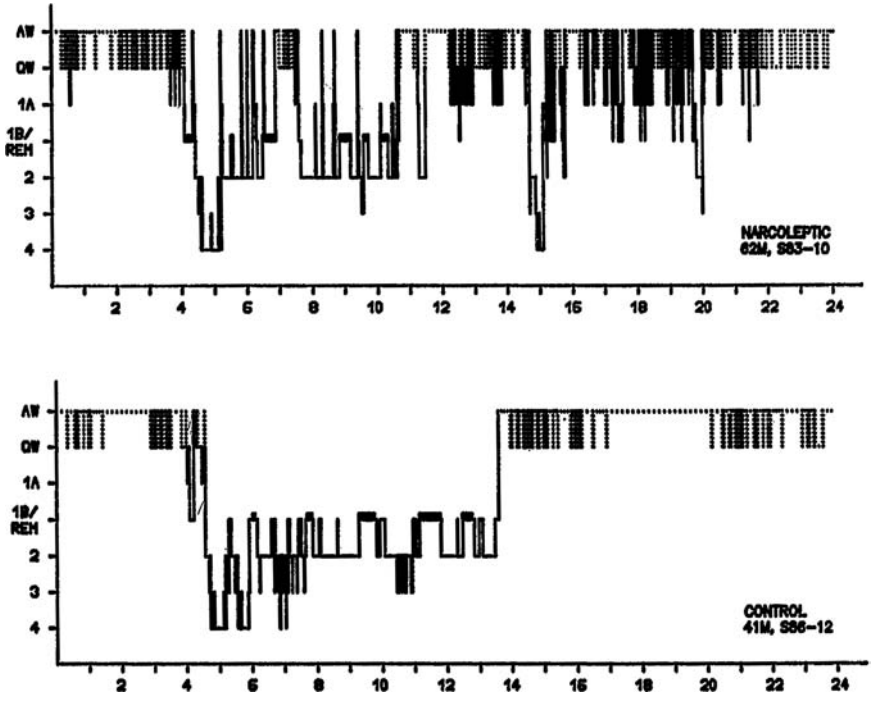


Figure 3 Histograms of the 24-hour sleep/wake status of a typical patient with narcolepsy compared to that of a typical normal control subject. The patient shows many more fluctuations between active wakefulness (AW), quiet wakefulness (QW) and sub-stages 1A and 1B than does the normal subject (below). An evening SOREMP, night sleep fragmentation and high amounts of day sleep in the patient with narcolepsy are also evident. (Time base is in hours after recording onset.)

longer and more boring situations are more likely to be associated with the more sustained waxing and waning patterns. Studies in our laboratory support this premise, as we found that during the long and boring 1-hour Wilkinson auditory vigilance task persons with narcolepsy spent over 50% of the time in waxing and waning patterns of mild drowsiness or light sleep and, moreover, that there were few microsleeps (14). By comparison, control subjects doing the same test remained fully awake 98% of the time. It remains uncertain whether the amnesic automatisms encountered so frequently in narcolepsy represent either repeated microsleeps or prolonged patterns of waxing and waning. In my estimation it is more likely to be the latter. To date there have been no reports of ambulatory monitoring during amnesic automatisms in narcolepsy to decide this issue.

Even longer duration episodes of sustained sleep lasting dozens of minutes to a couple of hours also characterize the daytime portion of physiological recordings in narcolepsy. These prolonged periods of sleep can either represent repeated involuntary sleep episodes ("sleep attacks"), which tend to be in the 5–20 minute range, or voluntary sleeps (naps), which are often much longer.

After Eugene Aserinsky's epochal discovery and full clinical and polygraphic description of REM sleep in the mid 1950s (15,16), there was an understandable early emphasis upon the daytime sleep onset REM periods (SOREMPs) found to characterize narcolepsy (17). However, it soon became evident in daytime recordings that onsets into both non-REM (NREM) and REM sleep are facilitated in narcolepsy/cataplexy syndrome.

The total amount of daytime sleep in narcolepsy varies greatly with a number of factors. These include: the recording methodology (short electrode wires, long cable, intensive monitoring by telemetry, ambulatory monitoring under real life conditions, etc.); the postural state (lying down, sitting, standing); environmental factors (ambient temperature, noise level, even in some patients the barometric pressure, etc.); the activity levels (quiet, moving while sitting down, walking, running, etc.); and the performance demands on the patient (none, minimal and semiautomatic, challenging, etc.), as well as the treatment status (none, wake promoters, psychomotor stimulants, tricylics, SSRIs, etc.).

As would be anticipated, considerably more daytime sleep is present for untreated patients lying in a hospital bed recorded by traditional polysomnography (18) than is the case for patients recorded during unrestricted daily work and household activities by ambulatory monitoring (19). In fact the latter approach shows no increase in amount of individual sleep stages or of total sleep per 24 hours in narcolepsy, other than a significant increase in stage 1 drowsiness (19). Posture has an effect not only on total daytime sleep but also on sleep stage distribution, as it has been shown that sitting compared to lying down greatly reduces the proportion of REM sleep in narcolepsy (20).

The very marked increase in daytime sleep propensity that characterizes narcolepsy is most commonly now assessed by consensus standardized sleep latency tests such as the MSLT and MWT. In MSLT studies of untreated patients with narcolepsy-cataplexy, about 50% of daytime naps exhibit SOREMPs, which is a proportion similar to their evening sleep onsets. The behavioral means of minimizing daytime sleepiness in narcolepsy are considered in a separate chapter of this volume (21).

Although the very short mean sleep latency on MSLT that characterizes untreated patients with narcolepsy is usually considered to reflect a heightened pressure for sleep, other explanations are equally plausible. It could for instance represent a facilitated sleep onset process with no increase in homeostatic sleep pressure. In any event, rapid entry into *either* NREM or REM sleep characterizes narcolepsy. This feature has led our center to question whether qualitatively different sleepy states might not exist immediately prior to entry into REM or into NREM sleep.

II. Do Qualitatively Different States of Sleepiness Exist?

It has generally been assumed that sleepiness is a homogeneous state that varies only in its intensity. However, just as it has now been widely recognized for four decades since the landmark paper of Snyder (22) that there are three qualitatively distinct biological states (wakefulness, NREM sleep and REM sleep), it seems quite possible that qualitatively different sleepy states might well exist. As awake patients with narcolepsy

enter with more or less equal frequency into NREM and REM sleep, they form an excellent model in which to assess for the possible existence of so-called "NREM sleepiness" and "REM sleepiness" immediately prior to those sleep states (23).

Our laboratory has explored this issue employing two types of complex evoked related potentials (ERPs). The first is the P300 paradigm, focusing mainly on component P3 which is associated with the detection of a stimulus change. The second is the Contingent Negative Variation (CNV), earlier referred to as the "expectancy wave" by its discoverer Grey Walter and others, in which a widespread DC negative shift maximum in the frontopolar areas and decreasing in amplitude posteriorly is emitted starting immediately after a warning stimulus (S1) and is sustained during the interval while awaiting a second stimulus (S2) that requires discrimination from a series of similar stimuli and then a motor response giving the reaction time (RT) to S2. These two ERPs along with the Stanford Sleepiness Scale were measured immediately prior to MSLT naps in untreated patients with narcolepsy and matched normal controls subjects (23).

The awake state immediately prior to REM sleep compared to that before NREM sleep was characterized by greater subjective sleepiness on the Stanford Sleepiness Scale, greater objective sleepiness shown by a shorter mean sleep latency on MSLT, a larger P2 component (a positivity with a latency around 200 msec maximum fronto-centrally) for both the P300 and CNV paradigms, a strong trend for a smaller P3 component (a positivity with latency around 300 msec maximum in parietal areas), and an essentially absent CNV. The group mean P300 paradigm ERPs are shown in Figure 4. On the other hand, the CNV immediately prior to NREM sleep did not differ significantly from that of sustained wakefulness in normal rested subjects. Although both subjective and objective sleepiness were greater in REM sleepiness prior to REM-containing naps, such naps had a greater recuperative effect on sleepiness as measured by the SSS after the naps than did NREM-only naps. Burton et al. (24) have confirmed that shorter sleep latencies characterize MSLT naps that are REM containing, compared to those that are NREM-only.

Alloway and colleagues have examined quantified EEG using spectral analysis (they chose spectral amplitude rather than spectral power [amplitude squared]) in the various EEG frequency bands immediately prior to NREM-only and REM-containing MSLT naps in narcolepsy (25). Although during REM sleepiness these authors found only a strong trend for a shorter mean sleep latency, they reported an enhanced spectral amplitude both in the delta and the theta bands, along with a reduced spectral amplitude in the alpha and sigma bands, during the sleep onset transition into REM-containing naps, compared to stage 1-only naps. An increase in spectral amplitude in the delta band also characterized the sleep onset period of REM naps when compared to stage 2-containing naps. The authors concluded that the spectral amplitude of the EEG during the sleep onset process is different prior to REM-containing naps than to prior to NREM-only naps (and whether stage 1-only, or stage 2-containing). In all these studies the physiological differences immediately before REM or NREM sleep are not marked with the exception for the striking reduction of CNV amplitude prior to REM sleep.

That there are qualitatively different types, or at least qualitatively different shades, of sleepiness should not be surprising. Studies of nocturnal sleep inertia in normal subjects immediately upon awakening from NREM and from REM sleep

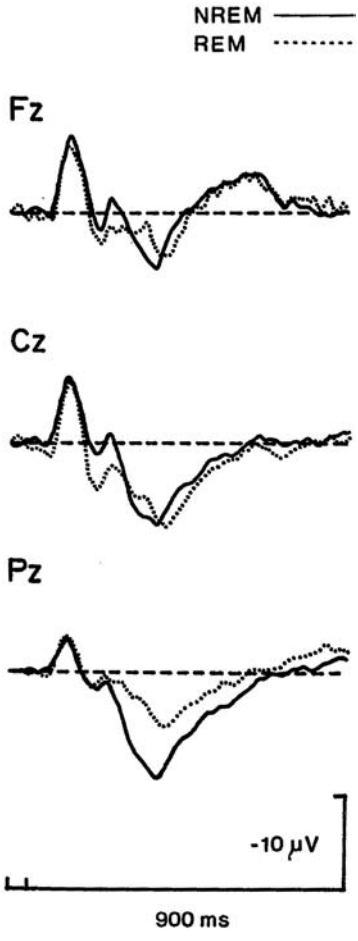


Figure 4 P300 paradigm done during the 10-min period immediately prior to NREM-only sleep and REM sleep in MSLT naps. Group average data are shown at the three midline electrodes referred to linked mastoids with positivity down. The first sizeable component is N2 (negativity at approximately 200 msec latency) which is essentially identical in the two states. The subsequent P2 (immediately subsequent positivity measured at CZ) is larger prior to REM sleep, whereas the subsequent larger and longer duration component P3 (positivity at approximately 300 msec latency component maximum at at measured at the Pz electrode) is smaller prior to REM sleep. *Source*: From Ref. 14.

show qualitative differences in ERPs, in level of conscious awareness, and in memory capacity (reviewed in 26). Moreover, the effects of sleep deprivation and recuperative oversleeping (with consequent "sleep satiation") produce qualitatively different subjective sleepiness states which are associated with a differential impact on dependent measures of performance. The latter can be encountered even within a single test such as the Wisconsin Card Sort Test (27).

III. Variations in Alertness/Drowsiness and Performance Vigilance in Narcolepsy

It is important to determine to what extent the fluctuations in alertness/drowsiness, as well as subjective daytime sleepiness independent of actually falling asleep, impact on the functional capacities of patients with narcolepsy. Studies have shown that even untreated narcolepsy patients are able to do some tasks (especially short challenging ones) quite normally, whereas other tasks, such as psychomotor vigilance tasks, are very impaired and no amount of cognitive effort will lead to normal performance levels.

The term vigilance, like that of arousal, is unfortunately employed with multiple meanings. The first is to denote general background level of CNS activation. The second is that of paying attention, that is, of being "vigilant." The third is the concept of Sir Henry Head, who introduced the term into medical psychology and who defined it as the efficiency of a system. This was true whether it be a relatively simple system such as the spinal cord reflexes in an isolated preparation, or a very sophisticated system such as occurs in complex cognition. Following Head, performance and cognitive psychologists, particularly those in the Cambridge tradition, generally restrict use of the phrase "vigilance tests" to describe and distinguish that group of tests in which very subtly different stimuli (called the "target stimuli" or, less appropriately, the "signal stimuli") must be detected in a train of similar stimuli.

Valley and Broughton (14) compared patients with narcolepsy off stimulants to matched controls on two short challenging tests, the Paced Auditory Serial Addition Task (PASAT) and the Digit Span test, as well on the 10-minute Wilkinson four-choice reaction time test and on the 1-hour Wilkinson auditory vigilance task. The latter test requires the very difficult detection of 40 slightly shorter (375 vs. 500 msec) tones (the "target stimuli") which occur pseudo-randomly within a continuous series of tones repeated every 2 seconds for 60 minutes with the tones being submerged in 85 db of background white noise. The test is designed to be so difficult that even fully rested normal subjects are on average only able to detect about 60% of the signal stimuli. It therefore excludes any possibility of a "ceiling effect."

Untreated patients with narcolepsy were able to do the PASAT and Knox Cube tests at normal levels despite a self-assessed marked increase in subjective sleepiness and higher self-assessed levels of effort. Moreover, polygraphic monitoring indicated that most patients were able to remain fully awake throughout both of these short challenging tests. On an equal duration (10 min) but more boring four-choice RT test, patients compared to controls showed more variable RTs and slower mean RTs, along with more gaps (responses >1000 msec, usually occasioned by inattention or microsleeps), whereas there was no increase in errors. Slowness of response was therefore traded for increased accuracy, a common strategy of sleepy persons. The 1-hour auditory vigilance task, however, was extremely poorly done by patients with narcolepsy, who overall detected many fewer signal stimuli than did the control subjects.

Subsequent retroactive detailed study of the correlation between subtle EEG changes and performance efficacy on the vigilance task (28) showed that performance capacity was exquisitely sensitive to even very minor levels of drowsiness as measured by EEG. The EEG pattern during the three-second mini-epoch when a signal stimulus happened to occur was first visually scored and then the detection rate was assessed. For target stimuli coincident with a three-second mini-epoch of wakefulness, patients with

narcolepsy detected 38% of stimuli, versus 57% for controls; and during the very minor drowsiness of stage 1A they detected many fewer, only 14%. Only two subjects responded in stage 1B; and none responded in stage 2.

Those signal stimuli coinciding with a three-second mini-epoch of wakefulness were then divided operationally into those in which the prior 10 seconds were also in wakefulness (referred to as "sustained wakefulness") and those in which the prior 10 seconds showed at least some drowsiness (referred to as "fragmented wakefulness"). Sustained wakefulness was associated with a level of performance (Fig. 5) that was not statistically different from controls (mean 47% vs. 59% of detections, NS), whereas in fragmented wakefulness the detection rate was only 18% (i.e., reduced by more than half) despite the EEG at the time of arrival of the signal stimuli being one of wakefulness.

This study is presented in some detail, as it shows the exquisite sensitivity of a signal detection vigilance task to generally ignored very subtle EEG changes. It also clearly demonstrates that, when the EEG shifts from drowsiness to one of full wakefulness, performance remains very impaired for some period of time. Stated differently,

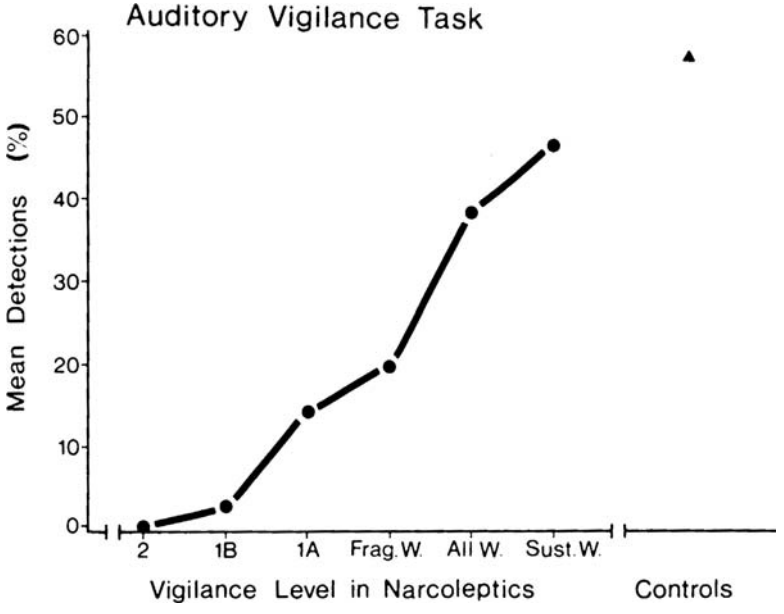


Figure 5 Performance on the long, boring and difficult 1-hour Wilkinson's auditory vigilance task in patients with narcolepsy and matched controls. The control subjects showed almost no drowsiness during the test and their overall detection rate of the slightly shorter target stimuli was 56%. In sustained wakefulness the patients with narcolepsy had a somewhat lower mean detection rate of 47%, but the difference was not statistically different. Fragmented wakefulness in which the preceeding 10 sec of recordings showed some degree of drowsiness, however, was associated with a marked deterioration of detection rate to 18% despite the fact that these stimuli fell within a 3 sec mini-epoch of EEG wakefulness. Detection rate for target stimuli was very low in stage 1B (stage 1 of Rechtschaffen-Kales) and was absent in stage 2 sleep.

there exists a "*drowsiness inertia*" effect on performance capacity which is equivalent to the well know sleep inertia effect following awakenings from true sleep including from naps in narcolepsy (29). The study furthermore underlines the crucial importance of being able to sustain wakefulness over time in order to be able to perform optimally. It would therefore appear that *wake continuity* is as important to fulfill the functions of wakefulness as is sleep continuity to fulfill the functions of sleep (30). Unfortunately a main problem for patients with narcolepsy is in fact exactly that—an inability to sustain wakefulness over time.

One of the related major performance deficits shown by patients with narcolepsy, and indeed by all very sleepy individuals, is a parallel great difficulty to sustain performance on sensitive tasks, a phenomenon often referred to as performance "fatiguability." It has been shown that there is already a significant decay of performance in the second 5 minutes of a 10-minute application of the four-choice reaction time test (31). In a 30-minute simulated driving task, George and colleagues (32) have shown that both narcolepsy and sleep apnea patients have a marked and increasing deterioration over time in their ability to keep the simulated virtual "car" on target.

To date no studies in narcolepsy have assessed performance immediately *prior* to REM-containing and NREM-only naps. Certainly for *non-initial* nocturnal SWS compared to REM awakenings there is typically a much poorer performance referred to as sleep inertia. There is, however, a study in patients with narcolepsy that assessed memory by giving materials prior to SOREMP-naps and NREM-only naps on MSLT and when there was no sleep (33). Recall of material was more complete after REM naps than after NREM naps, and both conditions improved recall more than the no nap condition. This could be predicted, as it is well known that REM sleep facilitates memory. Unfortunately this protocol does not make it possible to separate the effects of the sleepy state prior to naps from the effects of the sleep type during the naps. Proof of a differential effect of REM-sleepiness versus NREM-sleepiness on memory would require retesting for recall prior to the nap onset.

An important issue, little if at all considered in the literature, is whether persons with narcolepsy, or indeed sleepy persons in general, pay a price for a sustained attempt to fight off somnolence and drowsiness. Patients often say that forcing themselves to stay maximally awake for a period of time is followed by a period of enhanced sleepiness. This implies the existence of a phenomenon of waking "sleepiness homeostasis" complimentary to that of sleep homeostasis. It would seem a relatively easy matter to experimentally document any rebound reductions of arousal level after sustained efforts to maximize alertness in narcolepsy confirming the subjective reports during studies involving performance testing (14). To my knowledge this has not yet been reported. The results would have significant practical value for counseling in respect to predicting periods of enhanced sleepiness and of how to avoid them.

Cataplexy has been reported to be facilitated at the termination of a period of sustained intense wakefulness and indeed drowsiness itself facilitates the elicitation of cataplexy (34). In the previously mentioned study with Valley (14) it was noted that during the PASAT test (which was performed overall at normal levels), the narcolepsy patients rated both their effort and their subjective sleepiness much greater than did controls. Of the ten patients tested, eight showed EEG patterns of wakefulness throughout the PASAT, whereas two had considerable amounts of stage 1A immediately after terminating the test. The latter two patients, and only they, had an episode of cataplexy.

The issue is often raised as to whether the remarkable increase in subjective sleepiness and/or actual drowsiness in narcolepsy-cataplexy is not simply a marked accentuation of patterns seen in normal healthy persons under various circumstances. My current understanding is that the answer is "both yes and no." Certainly it appears that selective pressure for NREM or REM sleep, or pressure for sleep as a whole may be sufficiently increased by the selective or the predominant deprivation of SWS or REM sleep, or by total sleep deprivation, to show increased subjective (by SSS, ESS or 10 cm visual-analogue) and objective (by MSLT, MWT, daytime PSG, or sensitive performance tests) sleepiness/drowsiness into what is considered the pathological range; and SOREMPs can occur in normal subjects, particularly in the case of significant REM deprivation, that are similar to the situation in narcolepsy-cataplexy or in narcolepsy variants.

On the other hand, there are also situations which exhibit qualitative changes from normal sleepiness states. A clear-cut example of a variety of sleepiness more or less unique to narcolepsy is the pathological sleepiness associated with various forms of severe state dissociations such as often occurs in the context of status cataplecticus. In this condition, which is most often is seen with a too rapid withdrawal of patients from REM-depressant (or at least REM-altering substances) such as tricyclic, SSRI or MAOI "antidepressants," both the behavioral and polysomnographic data indicate that the patient can be simultaneously both partially awake and partially asleep in either REM sleep or NREM-drowsiness, or can slip rapidly in and out of wake/drowsy (REM or stage 1) sleep. It is even possible that at times some sectors of the brain are awake and others asleep as can occur in avian species or in cectaceans such as dolphins.

In any event, patients with narcolepsy-cataplexy in this fluctuating state of both conscious experience and motor phenomena (both of muscle tone and of fragmentary myoclonus of eyes, face, and peripherally) will often describe simultaneous and superimposed experiences of both perception of the environment and of what can only be described as hallucinations (if mainly awake) or dreams (if mainly asleep)—a phenomenon which can also occur at evening sleep onset. Elsewhere I have called this phenomenon "double consciousness" (35). Sleepiness or physiological drowsiness in this situation is, to my knowledge, never encountered in normal healthy subjects other than when exposed to hallucinogenic substances or similar toxins.

IV. What Is the Main Cause of the Marked Daytime Drowsiness Characterizing Narcolepsy?

As well as the fundamental cause of sleepiness in persons with narcolepsy, one must remember that there are often added supplementary causes which also require management by the physician. These include recent sleep deprivation (whether self-imposed or due to environmental or other factors) and the very frequent (about 25% of narcolepsy patients) coexistence of other sleep disorders such as sleep apnea (obstructive, mixed, or central), restless leg syndrome, periodic leg movements with or without arousals, or REM sleep behavior disorder (RBD). OSA appears to be increased due to the well-documented but still incompletely understood tendency of narcolepsy patients towards obesity; restless legs syndrome and/or periodic limb movements with arousals

are often due to treatment with "antidepressant" medications which amongst other actions reduce the normal atonia of REM sleep; and RBD can be due either to such medications or to the dissociations and fragmentation of REM sleep inherent in the condition. This section, however, concerns the fundamental cause of sleepiness/ drowsiness in narcolepsy and not the often superimposed ancillary causes.

In the evolution of the disease the marked sleepiness and physiological drowsiness that characterizes narcolepsy are typically the initial (36), and certainly the most persistent, symptoms; and indeed they remain for the life of the patient with some tendency to improvement in the later years. Moreover they are the most resistant symptom to treatment. Cataplexy, sleep paralysis and nightmares, by comparison, are intermittent symptoms. Significantly, it is to the unrelenting sleepiness/drowsiness that narcolepsy patients attribute their severe socio-economic and psychosocial problems (37). It is therefore crucial to determine the fundamental cause if one wishes to improve the quality of life and minimize the risk of accidents (sometime fatal) of these often quite handicapped individuals. Recent research findings have forced us to reconsider the etiology of pathological sleepiness in this condition.

The fundamental increase in daytime drowsiness and daytime sleep in untreated and treated narcolepsy have traditionally been considered to be secondary to increased sleep pressure due to night sleep fragmentation. This mechanism now seems highly unlikely indeed although it may add further to the underlying mechanism. The reasons for this reassessment of cause are several. During the disease onset process (whether auto-immune or other), daytime drowsiness and sleep episodes very often, and indeed usually, precede any significant fragmentation of night sleep and then by many months (36,37). Moreover, depending on the study, there is either absolutely no correlation (38), or only a very weak one (39), between the degree of prior night sleep fragmentation or its amount and the next day measures of sleepiness or sleep. Furthermore, in narcolepsy patients with very fragmented night sleep, improving its consolidation with hypnotics has little if any effect on either clinical assessment or objective daytime measures of sleepiness/ drowsiness (40).

The evidence now appears compelling that the daytime drowsiness and sleep in narcolepsy are not related primarily to sleep mechanisms per se; but rather they represent a weakness or insufficiency of daytime arousal systems, that is, an impairment in the mechanisms of wake maintenance, as proposed in the first international symposium on narcolepsy as a so-called "subvigilance syndrome" (41). Reduced waking arousal would readily explain the three above mentioned characteristics of the disease, that is, the frequent initial appearance at disease onset of daytime drowsiness and sleep a number of months or years before any change occurs in night sleep; the lack of correlation between daytime sleepiness levels and the amount or quality of the preceding night's sleep; and the relative inefficacy on daytime sleepiness of trying to improve night sleep quality by prescribing hypnotics. Two major findings in the past decade provide further strong support for this mechanism.

The first was the introduction by Bastugi and Jouvet of modafinil for the treatment of daytime sleepiness in narcolepsy (42). This is compelling evidence as modafinil in almost all clinical trials has been shown to alter night sleep little if at all and indeed it has essentially no effect on sleep even when taken by the elderly in the late evening (43). Unlike psychoactive stimulants such as methylphenidate and the amphetamines (from the first one introduced, ephedrine, to the current ones such as

dextro-amphetamine), use of modafinal does not lead to insomnia (44,45). Its bio-active effect is essentially restricted to enhancing wake-maintaining systems, although the exact mode of this action remains unclear. There is some evidence that modafinil can activate hypocretin-1 (orexin-A) neurons. These cell bodies are highly concentrated in the lateral hypothalamus, have widespread projections to the cortex, and are greatly decreased in numbers or essentially absent in narcolepsy-cataplexy both in the dog model (46) and in human narcolepsy (47).

The second relevant major discovery has been the finding that, at least in primates, the suprachiasmatic circadian pacemaker not only has a phase resetting function but also has an active arousal function. Lesions of the SCN in squirrel monkeys lead not only to random sleep/wake patterns demonstrating total loss of circadian pacemaking but also to a significant increase in amount of sleep per 24 hours (48).

There is evidence (49) that the afternoon so-called "nap zone" of transitory typically midafternoon sleep facilitation occurs due to accumulating sleep pressure with time after morning awakening, that is to process-S of Borbely (50), being reversed by this light sensitive SCN-dependent circadian arousal system and, which together sculpt the two/day circasemidian pattern of daily sleep propensity (49). Comparisons of 24-hour sleep propensity distributions in patients with narcolepsy and in normal habitual nappers (Fig. 6) have indicated that a weakened circadian arousal system exists (51) which would readily explain the striking 1 to 2 hour phase advance of the peak in daytime sleep propensity that characterizes narcolepsy (52–54), as well causing the overall increase in day sleep in the disease.

Since this discovery of the usual virtual absence of hypocretin-1 (orexin-A) neurones in the lateral hypothalamus of narcoleptic dogs (55) and of undetectable or very low hypocretin levels in the CSF or autopsied brains of patients with the disease (47,56,57), the issue has been raised as to whether this 33-aminoacid polypeptide is the sole or main neurotransitter or neuromodulator of daytime alertness in narcolepsy. This seems somewhat uncertain, as absence of hypocretin appears to be only detected when the REM based symptoms, in particular cataplexy, have appeared. Although it cannot be excluded that a minor loss of hypocretin neurons perhaps restricted to the hypothalamus could lead to daytime sleepiness and a greater loss of such neurons extending to other neural systems to the dissociations of REM sleep that typify the disease, it seems to this author that it is more likely that a separate neurochemical system is implicated. Evidence exists, for example, for diminished dopamine in the extrapyramidal system (mainly nigro-striatal) which represents a potential concurrent mechanism for problems sustaining wakefulness and that dopaminergic drugs may improve sleepiness in narcolepsy.

In fact the number of discrete systems that have been identified to subserve waking alertness are quite numerous, and any one or indeed a combination of them, might be impaired in the mechanism of the persistent drowsiness which characterizes narcolepsy. As well as the hypocretin (orexin) and dopamine systems, these other activating systems include the classical cholinergic reticulo-cortical system, the histaminergic posterior-hypothalamic system, and the noradrenergic locus coeruleus system, all of which have independent widespread ascending connections to the cortex. How these systems sustain different aspects of waking functions (e.g., overall alertness, attention, orientation, movement level, etc.), and interact hierarchically as the substrate of the many modulations of consciousness inherent in wakefulness, is

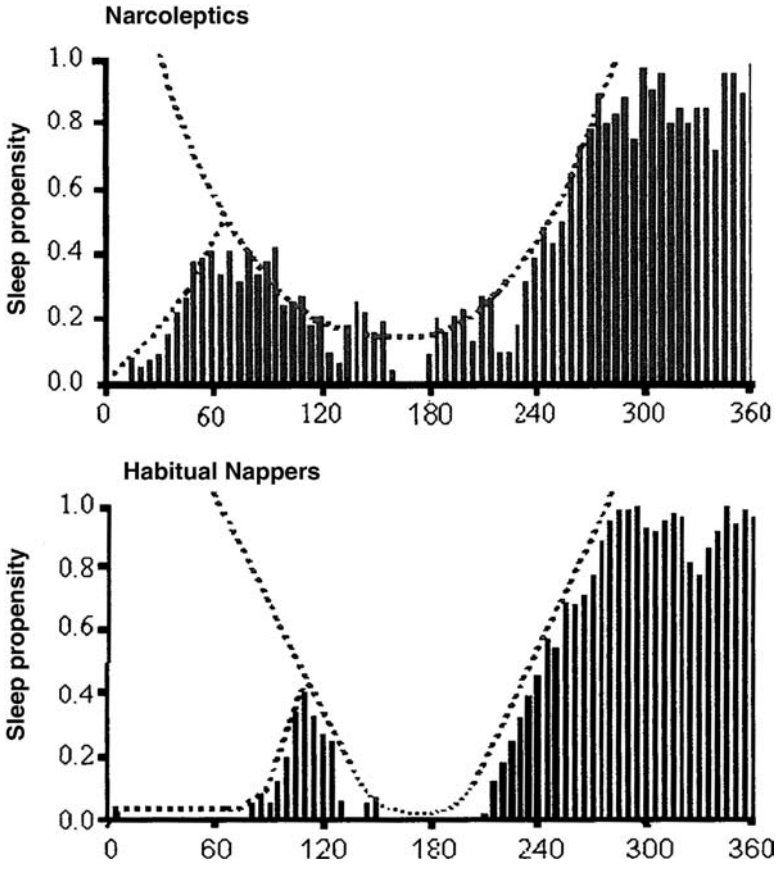


Figure 6 Comparison of sleep propensity distribution for untreated patients with narcolepsy-cataplexy compared to matched controls who were habitual nappers. The latter had a pattern of an afternoon nap recurring at least 5 days a week confirmed by a 3-week sleep log and by actigraphic monitoring. The vertical dimension represents the probability of sleep (1.0 = 100%) for each interval of time beginning at morning sleep onset (0 degrees). The dashed lines are fitted curves by polynomial regression up to the statistical average peak latency in the day sleep followed by a second fitted curve for the portion of sleep following the peak of daytime sleep. Patients with narcolepsy had more sleep in the daytime and less at night. Moreover, the peak of their daytime sleep was 70° after morning awakening versus 110° in normal nappers, a phase advance of 40° or 2.66 hours. The pattern is believed to represent process-S up to the peak of day sleep becoming temporarily reversed by the circadian arousal process (process-C) creating the nap zone which is quite brief in nappers and is much broader and earlier in narcolepsy. The findings are consistent with a very much weaker circadian arousal process in narcolepsy. *Source*: From Ref. 51.

not yet determined. But impairment or destruction of any of them, whether by brain lesion or chemically/pharmacologically, can lead to increased drowsiness.

This paper has focused upon the alterations in fully developed narcolepsy-cataplexy and space does not permit consideration of the narcolepsy variants. Whatever

fundamental mechanism underlies the striking diurnal hypoarousal that characterizes narcolepsy, a perhaps more fundamental issue is how this mechanism leads to the appearance of the dissociations of REM sleep subsystems giving rise to the symptoms of cataplexy and sleep paralysis. A large number of further types of dissociations have been described both between and within the states of wake, NREM sleep and REM sleep. These are discussed elsewhere (58) and include not only the dissociations of REM sleep that underpin the pathognomonic symptom of cataplexy and that of sleep paralysis, but also the frequent presence of intermediate or "mixed" stages of sleep, of REM bursts in NREM sleep, of brief episodes of REM atonia during NREM sleep, and a number of others. The existence of these many state component dissociations led to the proposal some two decades ago (58) that narcolepsy can perhaps most accurately be described as a disease or pathology of sleep/wake state boundaries rather than one of REM sleep. No new evidence exists that would incline me to alter this interpretation.

While basic research has done much to clarify the mechanisms controlling and expressing the three basic biological states, relatively little is known concerning those mechanisms (which must exist) that maintain the intactness or integrity of each state, that is, that act as a biological state or more precisely a state boundary "glue," and which appear to be defective in narcolepsy.

To summarize, the facts that in narcolepsy-cataplexy the REM-based symptoms almost never precede the development of daytime somnolence, do not explain the latter, and overall have much less impact on the quality of life of the patient, all underscore the great importance of our need to arrive at a more comprehensive understanding of the remarkable waking hypoarousal that characterizes narcolepsy.

Acknowledgments

The author thanks the Medical Research Council of Canada (now the Canadian Institutes of Health Research) for financial support throughout the period of the cited Ottawa studies. Particular recognition goes to my graduate students (in chronological order) Vicky Valley, Joel Hercovitch, Marissa Aguire, Janet Mullington, and Susanne Krupa whose doctoral research is extensively discussed in this chapter.

References

1. Daly DD, Yoss RE. Electroencephalogram in narcolepsy. Electroencephalogr Clin Neurophysiol 1957; 9:109–120.
2. Hishikawa Y, Kaneko Z. Electroencephalographic study on narcolepsy. Electroencephalogr Clin Neurophysiol 1965; 18:249–259.
3. Roth B. L'EEG dans la narcolepsie-cataplexie. Electroencephalogr Clin Neurophysiol 1964; 16:170–190.
4. Johnson L. Are sleep stages related to waking behavior? Amer Sci 1973; 61:326–328.
5. Gastaut H, Broughton R. A clinical and polygraphic study of episodic phenomena during sleep. Recent Adv Biol Psychiat 1965; 7:197–212.
6. Rechtschaffen A, Kales A, eds. A Manual of Standardized Technology, Techniques and Scoring System for Sleep Stages of Human Subjects. Washington: US Printing Office (Institute of Health Publications No. 204) 1968.

7. Loomis AL, Harvey EN, Hobart GA. Cerebral states during sleep, as studied by human brain potentials. J exp Psychol 1937; 21:127–144.
8. Oswald I. Sleeping and Waking. Amsterdam: Elsevier 1962.
9. Hori T. Spatiotemporal changes of EEG activity during waking-sleeping transitioin period. Int J Neurosci 1985; 27:101–114.
10. Simon O, Schulz H, Rassmann W. The definition of waking stages on the basis of continuous polygraphic recordings in normal subjects. Electroencephalogr Clin Neurophysiol 1977; 42:48–56.
11. Volk S, Simon O, Schulz H, Hansert E, Wilde-Frenz J. The structure of wakefulness and its relationship to daytime sleep in narcoleptic patients. Electroencephalogr Clin Neurophysiol 1984; 57:119–128.
12. Guilleminault C, Billiard M, Montplaisir J, Dement WC. Altered states of consciousness in disorders of daytime sleepiness. J Neurol Sci 1975; 26:377–393.
13. Tirunhari VL, Zaidi SA, Sharma R, Skurnick J, Ashtyani H. Microsleep and sleepiness: a comparison of multiple sleep latency test and scoring of microsleep as a diagnotic test for excessive daytime sleepiness. Sleep Medicine 2003; 4:63–67.
14. Valley V, Broughton R. Daytime performance deficits and physiological vigilance in untreated patients with narcolepsy-cataplexy compared to controls. Rev EEG Neurophysiol (Paris) 1981; 11:113–139.
15. Aserinsky E, Kleitman N. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. J Neuropsychiat Clin Neurosci 1953; 15:454–455.
16. Aserinsky E. The discovery of REM sleep. J Hist Neurosci 1996; 5:213–227.
17. Dement WC, Rechtschaffen A, Gulevich G. The nature of the narcoleptic sleep attack. Neurology 1966; 16:18–33.
18. Billiard M, Quera SalvaM, de Koninck J, Besset A, Touchon J, Cadilhac J. Daytime sleep characteristics and their relationships with night sleep in the narcoleptic patient. Sleep 1986; 9:167–174.
19. Broughton R, Dunham W, Newman J, Lutley K, Duschesne P, Rivers M. Ambulatory 24 hour sleep-wake monitoring in narcolepsy-cataplexy compared to matched controls. Electroencephalogr Clin Neurophysiol 1988; 70:473–481.
20. Hishikawa Y, Nan'no H, Tachibana M, Furuya E, Koida H, Kaneko Z. The nature of the sleep attack and other symptoms of narcolepsy. Electroencephalgr Clin Neurophysiol 1968; 41:1–10.
21. Broughton R, Murray B. The behavioral management of narcolepsy. In: C Bassetti, M Billiard, E Mignot, eds. Narcolepsy and Hypersomnia. New York: Marcel Dekker 2006: (this volume).
22. Snyder F. New biology of dreaming. Arch Gen Psychiatr 1963; 8:381–391.
23. Broughton R, Aguirre M. Differences between REM and NREM sleepiness measured by event related potentials (P300, CNV), MSLT and subjective estimate in narcolepsy-cataplexy. Electroencephalogr. Clin Neurophysiol 1987; 67:317–326.
24. Burton SA, Eastman CI, Kravitz HW. Factors relating to excessive daytime sleepiness in the narcolepsy syndrome. Sleep Res 1988; 17:154.
25. Alloway CED, Ogilvie RD, Shapiro CM. EEG spectral analysis of the sleep-onset period in narcoleptics and normal sleepers. Sleep 1999, 22:191–203.
26. Broughton RJ. Qualitatively different states of sleepiness. In: RJ Broughton, RD Ogilvie, eds. Sleep, Arousal and Performance: A Tribute to Bob Wilkinson. Boston: Birkhauser, 1992:45–62.
27. Herscovitch J, Stuss, Broughton R. Changes in cognitive processing following short term partial sleep deprivation and subsequent recovery oversleeping. J. Clin. Neuropsychol 1980; 20:301–319.
28. Valley V, Broughton R. Physiological (EEG) nature of drowsiness and its relation to performance deficits in narcoleptics. Electroencephalogr Clin Neurophysiol 1983; 55:243–251.
29. Mullington J, Broughton R. Daytime sleep inertia in narcolepsy-cataplexy. Sleep 1994; 17:69–76.
30. Bonnet M. The effect of sleep disruption on sleep, performance and mood. Sleep 1985; 8:9–11.
31. Godbout R, Montplaisir J. All-day performance variations in normal and narcoleptic subjects. Sleep 1986; 9:200–204.
32. George CFP, Bourreau AC, Smiley A. Comparison of simulated driving performance in narcolepsy and sleep apnea patients. Sleep 1996; 19:711–717.
33. Scrima L. Isolated REM sleep facilitates recall of complex associative information. Psychophysiology 1982; 19:252–259.
34. Parkes D, Chen SY, Clift SJ, Dahlitz MJ, Dunn G. The clinical diagnosis of the narcolepsy syndrome. J Sleep Res 1998; 7:41–52.
35. Broughton R. Human consciousness and sleep/waking rhythms: a review and some neuropsychological considerations. J Clin Neuropsychol 1982; 4:193–218.

36. Billiard M, Besset A, Cadihlac J. The clinical and poygraphic development of narcolepsy. In: C Guilleminault, E Lugaresi, eds. Sleep/Wake Disorders: Natural History, Epidemiology and Long-Term Evolution. New York: Raven, 1983:171–185.
37. Broughton R, Ghanem Q, Hishikawa Y, Sugita Y, Nevsimalova S, Roth B. Life-effects of narcolepsy in 180 patients from North America, Asia and Europe compared to matched controls. Can J Neurol Sci 1981; 8:299–304.
38. Broughton R, Dunham W, Weisskopf M, Rivers M. Night sleep does not predict day sleep in narcolepsy. Electroencephalogr Clin Neurophysiol 1994; 91:67–70.
39. Harsh J, Peszka J, Hartwig G, Mitler M. Night-time sleep and daytime sleepiness in narcolepsy. J Sleep Res 2000; 9:309–316.
40. Thorpy M, Goswami M. Treatment of narcolepsy. In: MJ Thorpy, ed. Handbook of Sleep Disorders. New York: Marcel Dekker, 1990:235–258.
41. Broughton, R. Discussion. In: C Guilleminault, WC Dement, P Passouant eds. Narcolepsy. New York: Spectrum, 1976:667.
42. Bastugi H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuropsychopharmacol Biol Psychiatr 1988; 12:695–700.
43. Saletu B, Frey R, Krupka M, Anderer P, Grunberger J, Barbanoj MJ. Differential effects of the new central adremergic agonist modafinil and d-amphetamine on sleep and early morning behavior in elderlies. Arzneim Forsch/ Drug Res 19890; 39:1268–1273.
44. Broughton RJ, Fleming JAE, George CFP, Hill JD, Kryger MH, Moldofsky H, Montplaisir JY, Morehouse RL, Moscovitch A, Murphy WF. Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. Neurology 1997; 49:444–451.
45. Modafinil US Narcolepsy Multicenter Study Group. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol 1998; 43:88–97.
46. Lin L, Faraco J, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder of canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–376.
47. Mignot E, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
48. Edgar DM, Dement WC, Fuller CA. The effect of lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. Physiol Behav 1993; 13:1065–1079.
49. Broughton RJ, Krupa S. Apparent mechanism of the afternoon "nap zone." Sleep, 2003, 26(Suppl): A96.
50. Borbely A. A two-process model of sleep regulation. Human Neurobiol 1982; 1:195–204.
51. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Research Online 1998; 1:159–165 (http://www.sro.org/1998/159/).
52. Mullington J, Newman J, Dunham W, Broughton R. Phase timing and duration of naps in narcolepsy-cataplexy: preliminary findings. In: J Horne, ed. Sleep'90. Bochum, UK: Pontanegal 1990:158–160.
53. Lavie P. REM periodicity under ultradian sleep/wake cycle in narcoleptic patients. Can J Psychol 1991; 45:185–193.
54. Pollak CP, Wagner D, Moline M, Monk T. Cognitive and motor performance of narcoleptic and normal subjects living in temporal isolation. Sleep 1992; 15:202–211.
55. Peyron C Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kutherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nature Medicine, 2000;6:991–997.
56. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron, 2000; 27:469–474.
57. Crocker A, Espana RA, Papadopoulou M, Saper CB, Faraco J, Sakurai T, Honda M, Mignot E, Scammell TE. Concomitant loss of dynorphin, NARP, and orexin in narcolepsy. Neurology, 2005; 65:1184–1188.
58. Broughton R, Valley V, Aguirre M, Roberts J, Suwalski W, Dunham W. Excessive daytime sleepiness and the pathophysiology of narcolepsy-cataplexy: a laboratory perspective. Sleep 1986; 9:205–215.

21
Molecular Characterization of Hypocretin/Orexin and Melanin Concentrating Hormone Neurons: Relevance to Narcolepsy

CHRISTELLE PEYRON
CNRS UMR 5167, Institut Fédératif des Neurosciences de Lyon, Université Claude Bernard-Lyon 1, Lyon, France

The link between hypocretin (Hcrt, orexin) neuropeptides and narcolepsy is now very well documented. Numerous animal models in mice, rats and dogs are available to confirm this relationship (1,2). Human narcolepsy is generally associated with a lack or a great reduction of preprohypocretin mRNA and Hcrt neuropeptides as shown by in situ hybridization (Fig. 1), radioimmunoassay and immunohistochemistry on brain and cerebrospinal fluid samples of narcoleptic patients at all ages (3–5). Based on these observations and the well-documented positive association of human narcolepsy with the human leucocyte antigen markers (6), the current hypothesis suggests that human narcolepsy is an autoimmune disease with hypocretin neurons as the target.

Although a dysfunction of the hypocretin system is responsible for narcolepsy, it is likely that the neurodegenerescence conducting to hypocretin cell death involves other molecules than the hypocretins themselves. We showed that Melanin Concentrating Hormone (MCH) neurons are intact in human narcoleptic patients (3). Therefore, the molecule targeted by the autoimmune attack should be expressed in Hcrt but not in MCH cells. Looking at the characteristics of these two neuronal populations is therefore critical.

I. Hypocretins/Orexins

The hypocretins are two peptides, Hcrt-1 (orexin-A) and Hcrt-2 (orexin-B), generated from a single preprohypocretin and synthesized by a small number of neurons restricted to the perifornical area of the hypothalamus (7). Hcrt axons are found throughout the central nervous system, with innervation of the hypothalamus, locus coeruleus, raphe nuclei, tuberomammilary nucleus, midline thalamus, cholinergic nuclei of the basal forebrain and the pons, all levels of spinal cord, sympathetic and parasympathetic centers, and many other brain regions (8,9). Since narcolepsy is characterized by an underlying disruption of the Hcrt system, it is likely that Hcrt participates in physiological sleep regulation and controls vigilance by modulating the activity of monoaminergic and cholinergic neurons.

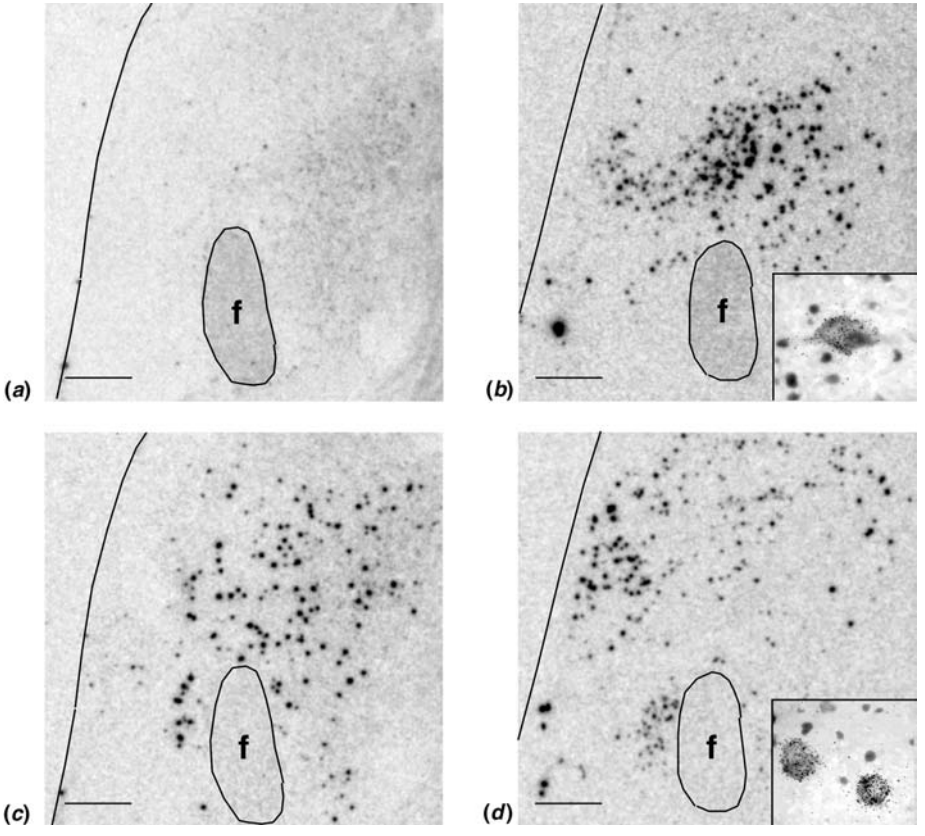


Figure 1 Hypocretin and MCH expression studies in the hypothalamus of control and narcoleptic subjects. *Preprohypocretin* transcripts are detected in the hypothalamus of control (***b***) but not narcoleptic (***a***) subjects. *MCH* transcripts are detected in the same region in both control (***d***) and narcoleptic (***c***) sections. *f*, fornix. Scale bar = 10 mm.

II. Melanin Concentrating Hormone

MCH neurons form an abundant cell population located in tuberal lateral hypothalamic area including the perifornical nucleus and the lateral hypothalamic area (10). This neuron population extends in rostromedial parts of the zona incerta, in the anterior, the posterior and the dorsomedial nuclei of the hypothalamus nucleus. Their widespread projections throughout the whole central nervous system suggest that MCH may act as a neurotransmitter or neuromodulator in a broad array of functions (10). Among its potential functions, the MCH neuronal system has been experimentally involved in the control of goal-oriented behaviors such as feeding, drinking or reproductive behaviors and stress responses (11). MCH neurons have been suspected to also play a role in arousal and modulation of memory. Expression of c-fos is particularly strong after paradoxical sleep rebound consecutive to a specific paradoxical

sleep deprivation (12). Intracerebroventricular injections of low doses of MCH induce an increase of paradoxical sleep and with a lesser extents of slow wave sleep (12) suggesting that MCH plays a role in homeostatic regulation of paradoxical sleep.

III. Co-Expression Data

The Hcrt and MCH neurons are medium in size (20–25 μm in diameter) (8). They are not identifiable based on their morphology but many antibodies are available on the market to specifically label them. Several studies have shown that Hcrt and MCH are expressed in two distinct but intermingled neuronal populations in rodents and human (3,8). However, the number of studies reporting co-expression for both, Hcrt or MCH neurons, is quite limited (Table 1).

A. Markers Common to Both Populations

Hcrt and MCH neurons are quite different based on their molecular characteristics, although they have some similarities. Indeed, both Hcrt and MCH express the serotoninergic receptor 5-$HT1_A$ (13) suggesting that the 5-HT hyperpolarizing effect of serotonin on Hcrt neurons is mediated through the 5-$HT1_A$ receptors. Hcrt and MCH neurons express the receptors GABA A-alpha 3 subunit, GABA A-epsilon subunit and GABA B (14–16). In addition, both neuronal populations express the acetylcholinesterase, an enzyme responsible for the degradation of acetylcholine (17,18).

We recently found that the neuropeptide cocaine-amphetamine-regulated-transcript (CART) is expressed in MCH neurons in rodents while it is present in 80% of the hcrt neurons in human as shown by in situ hybridization and double immunohistochemistry(19) (Fig. 2). The functional significance of the Hcrt/CART co-expression in human is difficult to grasp since only few human studies have been done. It is also difficult to attribute a role to the perifornical subpopulation of CART neurons in rodents because the CART neuronal population is heterogeneous. Nevertheless, it is of interest to note that CART peptide produces behavioral effects when injected into the VTA or the nucleus accumbens. In the VTA, the peptide behaves like an endogenous psychostimulant and produces increased locomotor activity and conditioned place preference. Since this is blocked by dopamine receptor blockers, it presumably involves release of dopamine (20).

Finally, it is interesting to note that neither Hcrt nor MCH expressed the hypocretin receptor 2 as shown by Volgin and colleagues using single-cell RT-PCR technique (21).

B. Molecular Characterization of Hcrt-Containing Neurons

Hcrt neurons are glutamatergic cells that express the group III metabotropic glutamate receptor and the vesicular transporter for glutamate vGlut1 and vGlut2 (22,23). They also express Narp, a secreted neuronal pentraxin implicated in regulating clustering of AMPA receptors (24). These data are in accordance with the fact that Hcrt 1 and Hcrt 2 were always found to be excitatory on neuronal population tested.

Hcrt neurons, but none of the MCH cells, express dynorphin and secretogranin II (25,26). The roles played by these proteins in Hcrt neurons are still unclear although they have been implicated in drinking and short-term feeding behaviors.

Table 1 Listing of Molecules Co-expressed in Hcrt and MCH Neurons in Rodents

	Hcrt	MCH	References
Neuropeptides			
Alpha MSH		Y (rodents) N (human)	(33)
CART	N (rodents but Y in human)	Y (rodents but N in human)	(19)
Dynorphine	Y	N	(25,26)
GAD	N	Y	(22,32)
Glutamate	Y		(37)
GRF-37		Y	(30)
MGOP		Y	(31)
NEI/NGE		Y	(10,30)
Secretogranin II	Y	N	(26)
Receptors and transporters			
5HT1-A	Y	Y	(13)
Adenose A1-R	Y		(29)
GABA A alpha 2	N	Y	(16)
GABA A alpha 3	Y	Y	(16)
GABA-A Epsilon	Y	Y	(15)
GABA B	Y	Y	(14)
Group III metabotropic glutamate receptors	Y		(23)
Hypocretin Receptor 2	N	N	(21)
Leptin-R	Y		(28)
NK-3	N	Y	(34)
VGLUT1	Y		(22)
VGLUT2	Y		(22)
Y4-R	Y		(27)
Others			
Acetylcholinesterase E	Y	Y	(18)
Narp	Y	N	(24)
NP1	N	Y	(24)
STAT-3	Y		(28)

Note: Hcrt and MCH neurons are two distinct neuronal populations of the tuberal hypothalamus. Both are heterogeneous expressing different type of molecules. Numbers refer to the concordant article's references. *Abbreviations*: CART, cocaine-amphetamine-regulated-transcript; GAD, glutamine acid decarboxylex; GRF-37, growth hormone releasing factor; MCH, Melanin concentrating hormone; MGOP, MCH-gene-overprinted-polypeptide; NEI, neuropeptide glutamic acid-isoleucineamide; NGE, neuropeptide glycine-glutamic acid.

Hcrt also express the receptor Y4 of the NPY neuropeptide (27). NPY is an orexigenic peptide that stimulate food intake although it does not induce c-fos expression in Hcrt neurons when injected in the lateral hypothalamus (27). The function of the Y4 receptor in Hcrt neurons is therefore unclear. In addition, Hakansson et al (28) have shown that Hcrt neurons express the leptin receptor and STAT3 the transcription factor activated by leptin (28). These results suggest that leptin may operate via an inhibitory action on neurons containing the excitatory peptide Hcrt resulting in a reduced food intake.

Thakkar et al. (29) have described that approximately 30% of Hcrt cells express A1 adenosine receptor. Adenosine is hypnogenic molecule that inhibits wakefulness promoting neurons via a postsynaptic action mediated through A1 adenosine receptor.

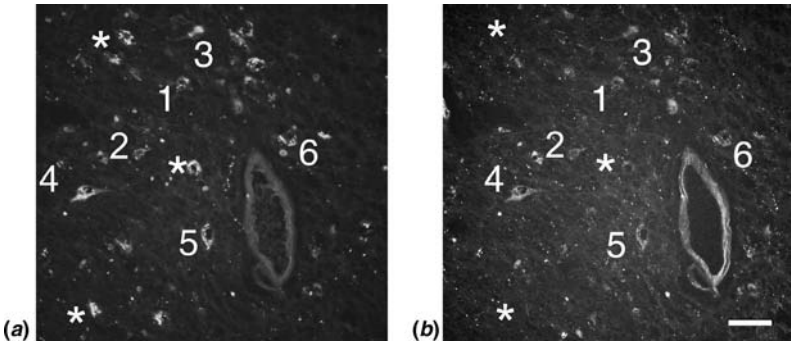


Figure 2 Illustration of neurons Hcrt (***a***)/CART (***b***) double labeled neurons in the perifornical area of the human hypothalamus as indicated by arrows. Note that most of the neurons co-express both peptides and only a few cells only express Hcrt as indicated by the asterisk (*). The specificity of antibodies has been tested in many controls. In the paraventricular nucleus of the hypothalamus containing a large number of CART cells but no Hcrt cells, no Hcrt labeling was found (data not shown). Scale bar = 120 μm.

C. Molecular Characterization of MCH-Containing Neurons

The MCH neuropeptide is co-expressed with the neuropeptide glutamic acid-isoleucineamide (NEI), the neuropeptide glycine-glutamic acid (NGE), and the MCH-gene-overprinted-polypeptide (MGOP) (10–31). NEI, NGE and MCH are encoded by a common precursor, the pro-MCH. The MGOP has been identified more recently by Nahon and co-workers (31). The MCH gene may produce, through alternative splicing, either the pro-MCH, precursor of MCH and NEI, or the MGOP. Based on its cerebral distribution, the authors suggested that MGOP may act as a new secreted protein regulating many neuroendocrine functions, such as nursing, feeding and growth control (31). However, the function of NEI and NGE is still unknown.

MCH neurons express GAD (22,32) and the receptor GABA-A alpha 2 subunit (16) in addition to those with a common expression in Hcrt cells as previously mentioned.

All MCH neurons co-express the growth releasing factor 1–37 (30). They also co-express alpha-melanocyte-stimulating-hormone (alpha MSH) in rat although they do not in human (33).

Furthermore, a majority of MCH neurons but none of the Hcrt cells express the NK3 receptors mediating the tachykinergic influence (34). Adrenergic alpha 2 mRNA were detected in most of the MCH neurons but none of the Hcrt neurons tested expressed it (21). The inhibitory effect of norepinephrine on Hcrt neurons may be thus mediated by another adrenergic receptor subtype.

Finally, MCH neurons express NP1 another neuronal pentraxin (24).

IV. Conclusion

Hcrt and MCH are quite different in their content although they are expressed in the same brain region and involved in the same function but in an opposite manner. A reciprocal interaction between these two neuronal populations has been shown in the

perifornical area by electron microscopy and in vitro electrophysiological recordings (35,36). Hcrt and MCH may therefore contribute to physiological regulation in harmony with each other through neural interactions.

Additional studies on the molecular characteristics of these neurons are surely needed. They would help to better understand the homeostatic regulation of the sleep-waking cycle through the hypothalamus and identify the mechanisms involved in the neurodegenerescence of hypocretin cells associated with human narcolepsy.

Acknowledgment

This work is supported by CNRS and Université Claude Bernard Lyon1.

References

1. Willie JT. Rodent models of narcolepsy. In: Bassetti C BC, Mignot E, ed. Narcolepsy and Hypersomnia, 2005.
2. Mignot E. Canine Narcolepsy. In: Bassetti C BC, Mignot E, ed. Narcolepsy and Hypersomnia, 2005.
3. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
4. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
5. Baumann CaM, E. CSF hypocretin-1/orexin-A studies, USA and European experience. In: Bassetti C BC, Mignot E, ed. Narcolepsy and Hypersomnia, 2005.
6. Lin L. HLA, present status. In: Bassetti C BC, Mignot E, ed. Narcolepsy and Hypersomnia, 2005.
7. de Lecea L. The hypocretins/orexins as integrators of physiological signals. In: Bassetti C BC, Mignot E, ed. Narcolepsy and Hypersomnia, 2005.
8. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18:9996–10015.
9. van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J Neurosci 1999; 19:3171–3182.
10. Bittencourt JC, Presse F, Arias C, et al. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 1992; 319:218–245.
11. Boutin JA, Suply T, Audinot V, et al. Melanin-concentrating hormone and its receptors: state of the art. Can J Physiol Pharmacol 2002; 80:388–395.
12. Verret L, Goutagny R, Fort P, et al. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci 2003; 4:19.
13. Collin M, Backberg M, Onnestam K, Meister B. 5-HT1A receptor immunoreactivity in hypothalamic neurons involved in body weight control. Neuroreport 2002; 13:945–951.
14. Backberg M, Collin M, Ovesjo ML, Meister B. Chemical coding of GABA(B) receptor-immunoreactive neurones in hypothalamic regions regulating body weight. J Neuroendocrinol 2003; 15:1–14.
15. Moragues N, Ciofi P, Lafon P, Tramu G, Garret M. GABAA receptor epsilon subunit expression in identified peptidergic neurons of the rat hypothalamus. Brain Res 2003; 967:285–289.
16. Backberg M, Ultenius C, Fritschy JM, Meister B. Cellular localization of GABA receptor alpha subunit immunoreactivity in the rat hypothalamus: relationship with neurones containing orexigenic or anorexigenic peptides. J Neuroendocrinol 2004; 16:589–604.
17. Risold PY, Fellmann D, Lenys D, Bugnon C. Coexistence of acetylcholinesterase-, human growth hormone-releasing factor(1–37)-, alpha-melanotropin- and melanin-concentrating hormone-like immunoreactivities in neurons of the rat hypothalamus: a light and electron microscope study. Neurosci Lett 1989; 100:23–28.
18. Chou TC, Rotman SR, Saper CB. Lateral hypothalamic acetylcholinesterase-immunoreactive neurons co-express either orexin or melanin concentrating hormone. Neurosci Lett 2004; 370:123–126.

19. Peyron C, Charnay Y. [Hypocretins/orexins and narcolepsy: from molecules to disease]. Rev Neurol (Paris) 2003; 159:6S35–6S41.
20. Jaworski JN, Vicentic A, Hunter RG, Kimmel HL, Kuhar MJ. CART peptides are modulators of mesolimbic dopamine and psychostimulants. Life Sci 2003; 73:741–747.
21. Volgin DV, Swan J, Kubin L. Single-cell RT-PCR gene expression profiling of acutely dissociated and immunocytochemically identified central neurons. J Neurosci Methods 2004; 136:229–236.
22. Rosin DL, Weston MC, Sevigny CP, Stornetta RL, Guyenet PG. Hypothalamic orexin (hypocretin) neurons express vesicular glutamate transporters VGLUT1 or VGLUT2. J Comp Neurol 2003; 465:593–603.
23. Acuna-Goycolea C, Li Y, Van Den Pol AN. Group III metabotropic glutamate receptors maintain tonic inhibition of excitatory synaptic input to hypocretin/orexin neurons. J Neurosci 2004; 24:3013–3022.
24. Reti IM, Reddy R, Worley PF, Baraban JM. Selective expression of Narp, a secreted neuronal pentraxin, in orexin neurons. J Neurochem 2002; 82:1561–1565.
25. Chou TC, Lee CE, Lu J, et al. Orexin (hypocretin) neurons contain dynorphin. J Neurosci 2001; 21:RC168.
26. Bayer L, Mairet-Coello G, Risold PY, Griffond B. Orexin/hypocretin neurons: chemical phenotype and possible interactions with melanin-concentrating hormone neurons. Regul Pept 2002; 104:33–39.
27. Campbell RE, Smith MS, Allen SE, Grayson BE, Ffrench-Mullen JM, Grove KL. Orexin neurons express a functional pancreatic polypeptide Y4 receptor. J Neurosci 2003; 23:1487–1497.
28. Hakansson M, de Lecea L, Sutcliffe JG, Yanagisawa M, Meister B. Leptin receptor- and STAT3-immunoreactivities in hypocretin/orexin neurones of the lateral hypothalamus. J Neuroendocrinol 1999; 11:653–663.
29. Thakkar MM, Winston S, McCarley RW. Orexin neurons of the hypothalamus express adenosine A1 receptors. Brain Res 2002; 944:190–194.
30. Risold PY, Fellmann D, Rivier J, Vale W, Bugnon C. Immunoreactivities for antisera to three putative neuropeptides of the rat melanin-concentrating hormone precursor are coexpressed in neurons of the rat lateral dorsal hypothalamus. Neurosci Lett 1992; 136:145–149.
31. Toumaniantz G, Ferreira PC, Allaeys I, Bittencourt JC, Nahon JL. Differential neuronal expression and projections of melanin-concentrating hormone (MCH) and MCH-gene-overprinted-polypeptide (MGOP) in the rat brain. Eur J Neurosci 2000; 12:4367–4380.
32. Dallvechia-Adams S, Kuhar MJ, Smith Y. Cocaine- and amphetamine-regulated transcript peptide projections in the ventral midbrain: colocalization with gamma-aminobutyric acid, melanin-concentrating hormone, dynorphin, and synaptic interactions with dopamine neurons. J Comp Neurol 2002; 448: 360–372.
33. Pelletier G, Guy J, Desy L, Li S, Eberle AN, Vaudry H. Melanin-concentrating hormone (MCH) is colocalized with alpha-melanocyte-stimulating hormone (alpha-MSH) in the rat but not in the human hypothalamus. Brain Res 1987; 423:247–253.
34. Griffond B, Ciofi P, Bayer L, Jacquemard C, Fellmann D. Immunocytochemical detection of the neurokinin B receptor (NK3) on melanin-concentrating hormone (MCH) neurons in rat brain. J Chem Neuroanat 1997; 12:183–189.
35. Guan JL, Uehara K, Lu S, et al. Reciprocal synaptic relationships between orexin- and melanin-concentrating hormone-containing neurons in the rat lateral hypothalamus: a novel circuit implicated in feeding regulation. Int J Obes Relat Metab Disord 2002; 26:1523–1532.
36. van den Pol AN, Acuna-Goycolea C, Clark KR, Ghosh PK. Physiological properties of hypothalamic MCH neurons identified with selective expression of reporter gene after recombinant virus infection. Neuron 2004; 42:635–652.
37. Torrealba F, Yanagisawa M, Saper CB. Colocalization of orexin a and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats. Neuroscience 2003; 119:1033–1044.

22
Nocturnal Polysomnography, Multiple Sleep Latency Test and Maintenance of Wakefulness Test in Narcolepsy

MERRILL M. MITLER
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Rockville, Maryland, U.S.A.

I. Summary

This chapter outlines the status of nocturnal polysomnography, the multiple sleep latency test (MSLT) and the maintenance of wakefulness test (MWT) in the evaluation and management of Narcolepsy. Much of the material discussed comes from the author's participation in the work of a task force appointed by The American Academy of Sleep Medicine. The taskforce concluded that nocturnal polysomnography is useful in the evaluation of patients for possible narcolepsy to assess for the presence and degree of other sleep disorders such as sleep apnea. In healthy controls and narcolepsy patients, mean sleep latency (MSL) on the MSLT and the MWT is sensitive to conditions expected to increase sleepiness. However, large between subject variance in MSL makes it difficult to establish a specific threshold value for excessive sleepiness or to discriminate patients with sleep disorders from nonpatients. Sleep latencies can detect change from initial testing to subsequent testing following treatment or manipulations intended to alter sleepiness or alertness. The presence of two or more sleep onset REM periods (SOREMPs) on the MSLT is a common finding in narcolepsy patients. But, SOREMPs are not exclusive to narcolepsy patients. They are found in other conditions such as untreated sleep apnea. The MSL is sensitive to circadian changes but a relationship between MSL and evaluation of safety in real life operations has not been established. A diagnosis of narcolepsy should be made cautiously and with complete clinical information. It may be necessary to go beyond electrophysiological approaches, to develop clinical laboratory tests for biomarkers in bodily fluids or other types of specimens.

II. Introduction

Seeds of present day practices for the polysomnographic evaluation of patients with the possible diagnosis of narcolepsy can be found in the proceedings of the First International Symposium on Narcolepsy (1). Dement's paper at that symposium pointed out that early EEG studies of patients with the complaint of excessive daytime

sleepiness were contradictory and did not lead to accepted diagnostic categorizations (2). Dement identified Vogel's 1960 study (3) as the first polysomnographic evaluation of patients with narcolepsy in which REM sleep and NREM sleep could be distinguished and the first to describe the phenomenon of sleep-onset REM periods in narcoleptics. The sleep onset REM period in nocturnal sleep of narcoleptic patients was later characterized by the independent studies of Rechtschaffen et al. (4) and Takahashi and Jimbo (5) Vogel's paper (6) described a protocol of using a 90-minute sleep opportunity beginning at approximately noon to document sleep-onset REM periods in patients with narcolepsy. Carskadon's paper (7) described data from normal subjects in the 90-minute day protocol that called for round-the-clock cycles of 60 minutes of enforced wakefulness and 30 minutes of ad libitum sleep. Thus, with the descriptions at the first symposium of (*i*) the phenomenon of sleep onset REM periods in narcolepsy, (*ii*) the reliable capture of such REM periods in daytime naps, and (*iii*) the 90-minute day protocol's standardized conditions for multiple polysomnographic recordings of daytime sleep opportunities, the MSLT was soon to be devised and employed diagnostically in narcolepsy and other conditions characterized by the symptom of excessive daytime sleepiness (EDS).

As we participate in this Fifth International Conference on Narcolepsy, we recognize that an important part of evaluation and management of the sleep disorders patient is the objective assessment for the presence and degree of EDS. Nocturnal polysomnography, The MSLT (8) and the MWT (9) have become the tests most often used for the objective measurement of nighttime sleep as well as daytime sleepiness and alertness. Results of these tests are used to help diagnose sleep disorders, evaluate treatment and to support recommendations to the patient on the advisability of driving or performing other daily activities. Daytime testing with the MSLT or the MWT have also been recommended by regulatory agencies to support a decision to return a sleep disorders patient to service in a safety-sensitive position after treatment.

This chapter summarizes data from polysomnographic evaluations of patients with possible diagnosis of narcolepsy and is based on exhaustive reviews, reports and guidelines formulated under the auspices of the Standards of Practice Committee of the American Sleep Disorders Association (ASDA) (10–12). Whenever possible, conclusions in these publications were based on published evidence. Where scientific data are absent, insufficient, or inconclusive, recommendations were based on consensus of opinion (10).

Guidelines for the use of nocturnal polysomnography were set-forth in 1997 (10). Guidelines for the use of the MSLT were initially established in 1992 based on consensus (13). Since then, the body of scientific literature using the MSLT and MWT has grown substantially. The Standards of Practice Committee of the American Academy of Sleep Medicine (AASM) requested an exhaustive review of the literature and a protocol-directed, quantitative evaluation of published data on the use of the MSLT and the MWT in research and clinical setting (12).

It is beyond the scope of this paper to discuss the relationships between objective measures of sleep and daytime sleepiness versus the various self-assessment tools in use such as the Stanford Sleepiness Scale (SSS) (14), the Epworth Sleepiness Scale (15) and the Karolinska Sleepiness Scale (16). Suffice it here to say that the correlations between objective and subjective measures of sleep in clinical situation have rarely reached a high degree of even statistical, let alone clinical, significance regardless of

how they are estimated (see for example Cook et al. (17)). This point is not meant to criticize the usefulness of subjective measures of sleepiness, only to state current circumstances. And, as will be discussed herein, the correlations between the two principal objective measures of sleepiness are rarely above 0.5 (see for example Sangal et al. (18)). Analogously, this point is not meant to criticize the usefulness of objective measures of sleepiness, only to state current circumstances.

III. Nocturnal Polysomnography

The American Academy of Sleep Medicine (10) advises that polysomnography be routinely used for the diagnosis of sleep-related breathing disorders; for continuous positive airway pressure (CPAP) titration in patients with sleep-related breathing disorders; for documenting the presence of obstructive sleep apnea in patients prior to laser-assisted uvulopalatopharyngoplasty; for the assessment of treatment results in some cases; with a MSLT in the evaluation of suspected narcolepsy; in evaluating sleep-related behaviors that are violent or otherwise potentially injurious to the patient or others; and in certain atypical or unusual parasomnias. Polysomnography may be indicated in patients with neuromuscular disorders and sleep-related symptoms; to assist with the diagnosis of paroxysmal arousals or other sleep disruptions thought to be seizure-related; in a presumed parasomnia or sleep-related epilepsy that does not respond to conventional therapy; or when there is a strong clinical suspicion of periodic limb movement disorder. In connection with the evaluation of patients with narcolepsy, nocturnal polysomnography is important for ruling-out the presence of significant sleep apnea and restricted sleep.

The most extensive nocturnal polysomnographic data in narcolepsy come from two clinical trials on the treatment efficacy of modafinil (19,20) and are reported by Harsh et al. (21). In a sample of 529 patients meeting the International Classification of Sleep Disorders criteria for diagnosis of narcolepsy, sleep data were obtained from polysomnographic recordings on two consecutive nights. Sleepiness was assessed using the MSLT, the MWT and the Epworth Sleepiness Scale. Analysis revealed that sleep was mild to moderately disturbed on both recording nights. A first-night effect was suggested by decreased REM latency and increased percentage REM and slow-wave sleep on the second night. Sleepiness and sleep disturbance varied across patient subgroups based on patient ethnicity and on the presence/absence of cataplexy, sleep apnea, and periodic limb movements. Covariation of sleep and sleepiness measures across patients was significant but weak. Strong association was found between subgroup means of sleep and sleep disturbance measures. The authors concluded that sleepiness and sleep disturbance vary across patient subgroups and that sleep disturbance is related to, although unable to account, for the pathological sleepiness of narcolepsy. For purposes of this chapter, selected data from the first night of these two narcolepsy clinical trails were compared with comparable data from a sample of 64 healthy controls from a multi-site project to establish normative values for the MWT (22). The comparisons generally confirm the observations of Harsh et al. with the exception that sleep efficiency was not significantly lower in narcolepsy relative to healthy control subjects.

	Narcolepsy $N = 529$ (238 M, 291 F) Ages: 18–68	SD	Control $N = 64$ (27 M, 37 F) Ages: 30–69	SD	*t*-test	$p <$
Total Sleep Time	396.70	65.70	416.70	63.13	−2.31	**0.025**
Sleep Efficiency	86.90	9.70	87.00	9.00	−0.08	ns
REM Latency	47.00	49.60	106.62	54.24	−8.99	**0.005**
Percent REM	21.70	7.30	19.40	4.98	2.45	**0.025**
Percent SWS	13.00	9.50	16.68	8.47	−2.96	**0.005**

IV. The Multiple Sleep Latency Test

The MSLT is thought to measure physiological sleep tendency in the absence of alerting factors (8) and is based on the assumption that physiological sleepiness decreases sleep latency. The MSLT procedure was formalized in 1977 to measure sleepiness in young normal subjects involved in sleep deprivation experiments (23,24). Six volunteers (age 18–21) underwent two nights of total sleep deprivation. They were put in bed and told to try to fall asleep every two hours during the wake period. Each test was terminated after 20 minutes if the subject did not fall asleep. If sleep occurred, the subject was awakened after one minute of stage 1 and the nap terminated to prevent accumulation of sleep during the sleep deprivation procedure. Sleep latency was measured from lights out to the first minute of stage 1. Significant correlations between the degree of deprivation and sleep latency gave face validity to using sleep latency as a biologically based measure of sleepiness. The MSLT was adapted to the evaluation of narcolepsy so that 10 min of sleep was permitted (25,26). The frequent occurrence of REM sleep in narcolepsy patients during the MSLT suggested a relationship between SOREMPs and narcolepsy (4,5). The perceived usefulness of the MSLT quickly expanded to include evaluation of various sleep disorders (27,28) effects of treatment (29,30) and effects of hypnotic medications (31).

The use of the MSLT for clinical and research purposes led to the development of a standardized diagnostic MSLT protocol (32). Separate clinical and research protocols were established. The research protocol was designed to limit the amount of sleep that a subject is permitted by terminating each MSLT nap after some prespecified criterion for sleep onset has been met. The clinical version of the MSLT was designed to allow each MSLT nap to progress to a prespecified termination time (15 min after sleep onset) in order to permit an opportunity for REM sleep to emerge. This "permit to sleep" feature is fundamental in the clinical use of the MSLT and figures importantly in the differential diagnosis of conditions associated with the symptom of excessive sleepiness, including narcolepsy.

It has recently been suggested that sleep latency is also a measure of one's ability to transition into sleep. This characteristic has been referred to as "sleepability" (33). Young adults with normal alertness and MSL values in the 6–8 minute range even after two weeks with time in bed increased to 10 hours have previously been identified as having "high sleepability without sleepiness" (33). Several studies have documented that patients with psychophysiological insomnia, who have decreased sleep at night,

actually have MSL values that are significantly longer ("low sleepability") than those of matched control subjects (34–36). Additionally, a significant negative correlation has been reported between total sleep time at night and MSL values on the following day (34–36). The finding that shorter prior sleep time is associated with longer MSLT latencies is the opposite of what would be expected if the MSLT were only sensitive to prior total sleep. Additionally, studies have shown that MSL is sensitive to both state and trait levels of central nervous system arousal (37).

The Task Force concluded that the MSLT was a valid and reliable test. It has been shown to be sensitive to sleep loss or sleep disruption and because with the 4-nap MSLT protocol, test-retest reliability was about 0.97.

Nine articles involving 255 patients with narcolepsy using the MSLT in narcolepsy were judged free of the inclusion bias, such as a low MSL on a diagnostic MSLT, were combined to disclose a MSL of 3.1 $\pm$ 2.9. From seven data sets involving the MSLT protocol that permits sleep to continue for 15 minutes (clinical protocol), the MSL was 11.0 with as SD of 3.4 minutes. Using a 5-minute cut point, about 16% of narcolepsy patients would have a MSL above this cutoff while about 16% of normal control subjects would fall below this cutoff (12).

Figure 1 summarizes a representative body of MSLT data combined from the modafinil clinical trials in patients with narcolepsy and comparable data from a representative sample of healthy control subjects (19,20,38).

While the difference between the patient and control groups in Figure 1 are statistically significant (all p's $< .001$), the Task Force concluded that current data make difficult, the use of the MSLT to categorize individual patients, or control subjects, as pathologically sleepy because MSLT MSLs range from 0 to 14.6 for the narcolepsy patients and from 2 to 18 for control subjects.

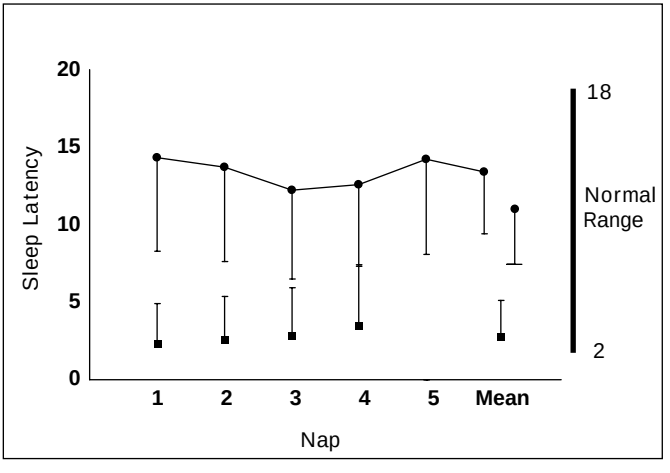


Figure 1 Summary of a representative body of MSLT data combined from the modafinil clinical trials in patients with narcolepsy (squares) and comparable data from a representative sample of healthy control subjects (circles).

V. Sleep Onset REM Periods

The MSLT reliably differentiates groups of patients with narcolepsy from control subjects in terms of the number of REM sleep periods achieved on the naps (SOREMPS). In ten studies that met task force evaluation criteria, data showed that the majority of narcolepsy patients have two or more SOREMPs. The presence of SOREMPs was not linked to the presence of cataplexy. SOREMPS were also found frequently in patients with OSA. Among patients with narcolepsy, the number of SOREMPs was found to increase with decreasing MSL on the MSLT. Analysis of the articles with appropriate data showed a sensitivity of 0.79 and a specificity of 0.98. However, the Task Force concluded that SOREMPS can be seen in other patient groups such as those with sleep apnea and those who are sleep deprived or have irregular sleep and wake patterns.

VI. The Maintenance of Wakefulness Test

The MWT is thought to measure the ability to stay awake under soporific conditions for a defined period of time (9). The MWT rationale is based on the assumption that the volitional ability to stay awake is more important to know in some instances than the tendency to fall asleep. Since there is no direct biological measure of wakefulness available, this same phenomenon is calibrated indirectly by the inability or delayed tendency to fall asleep, as measured by the same EEG-derived initial sleep latency employed in the MSLT. In a large study of patients with sleep disorders, MWT sleep latencies were found to have a low but statistically significant correlation with the MSL on the MSLT (18). The low correlation may reflect the combination of underlying sleepiness and motivational arousal present in the MWT. The MWT procedure calls for subjects to sit up in a chair in a quiet and dimly lit room with instructions to stay awake. Vocalizations and movements were not allowed. Four or five trials were given beginning 1.5 to 3 hours after awakening and recurring every two hours thereafter. Each trial was terminated after 20 minutes if no sleep occurred or immediately after sleep onset. It was reasoned that instructing patients to stay awake rather than to allow themselves to fall asleep was a more accurate reflection of their ability to function and maintain alertness in common situations of inactivity. Subsequent studies demonstrated significant pre- and post-treatment differences in initial sleep latencies on the MWT in patients with excessive somnolence (39). Using data from the two large clinical trials on modafinil in narcolepsy, the MSL on the MWT is observed to have a sensitivity of 84% and a specificity of 98% using a cutoff of <12 minutes (40).

Figure 2 summarizes a representative body of MWT data gleaned from the two clinical trials on modafinil in narcolepsy (19,20) and the MWT normative study (22).

As with the MSLT, between-group differences between patients with narcolepsy and controls are statistically significant. However, the ranges of the two groups do overlap and it is difficult to use the MWT to categorize individual patients, or control subjects, as pathologically sleepy because MWT MSLs range from 0 to 20 for the narcolepsy patients and 7.5 to 20 for control subjects.

Analysis of normative data for both the MSLT and the MWT showed that there are many methodological factors that affect MSL. Four factors that most powerfully

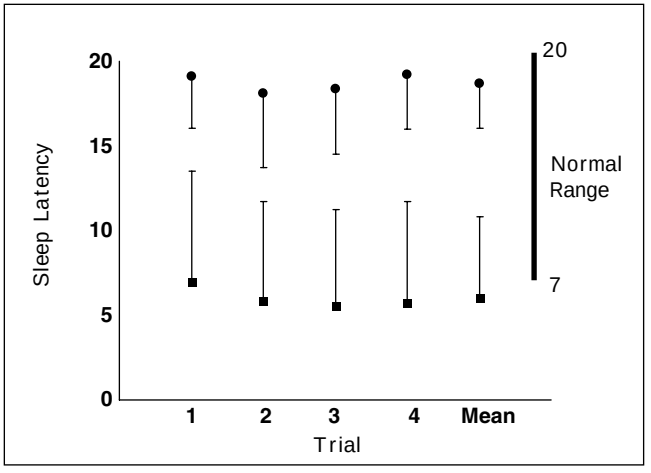


Figure 2 Summary of MWT data gleaned from the two clinical trials on modafinil in narcolepsy (squares) (18,19) and the MWT normative study (circles). *Source:* From Ref. 21.

affected the MSLT were: number of naps, age, sleep latency definition, and prior TST. Type of protocol (research or clinical) did not result in significant differences in MSL. The significant effect for number of naps demonstrated longer latencies with a five nap protocol due to a more prominent "last nap effect." Age effects included longer latencies for 50 and 80 year old age groups compared to all younger age groups. (A significant age effect was also found for the MWT). The significant difference due to sleep latency definition showed that latency to the first epoch resulted in a significantly shorter MSL than the latency to sustained sleep; however the 0.7 min difference is probably not clinically significant. Finally, prior TST was inversely related to MSL showing that shorter TST (mean of 6.6 hrs) resulted in longer MSL than longer TST (mean of 7.5 hours). This suggests that there is a trait component to sleep latency and that short normal sleepers are not necessarily sleep deprived. However, when normal sleepers are sleep deprived, the MSL is decreased. Given all the factors that affect MSL it is not possible to determine one number to represent a normal control mean and SD for the MSLT. At a minimum, MSL comparisons should be made to data from a similar age group and for the number of nap opportunities allowed.

VII. Conclusion

This chapter has summarized data relevant to daytime and nighttime polysomnographic evaluation of narcolepsy from the work of a task force charged by The American Academy of Sleep Medicine with performing a thorough literature review. The task force concluded that mean sleep latency on the MSLT and the MWT is sensitive to conditions expected to increase sleepiness. Mean sleep latencies are generally lower following sleep loss, following use of sedating medications, during wakefulness in the late night or early morning hours, and among patients with sleep disorders associated with excessive sleepiness such as narcolepsy or obstructive sleep apnea. However,

the wide range in MSL makes it difficult to establish a specific threshold value for excessive sleepiness. Some of this variation may be attributable to methodological differences and some may be attributable to individual differences in sleep tendency.

The MSL on both the MSLT and MWT does not discriminate well between individuals with sleep disorders and normals. This is due, not only to the large between subject variance, but also to floor and ceiling effects. However, the MSL shows appropriate change from initial testing to subsequent testing following treatment or manipulations intended to alter sleepiness or alertness. Additionally the presence of two or more SOREMPs on the MSLT is a common finding in narcolepsy patients. However, SOREMPs are not exclusive to narcolepsy patients but are frequent in untreated sleep apnea patients. This underscores the necessity of ruling out or treating other sleep disorders before interpreting SOREMPs for diagnostic purposes. Finally, the MSL is sensitive to circadian changes but a relationship between MSL and evaluation of safety in real life operations has not been established. Therefore, a diagnosis of narcolepsy should be made cautiously and with as much clinical information as possible. Based on current evidence, the MSL should not be the sole criterion for determining sleepiness or for certifying a diagnosis or response to treatment. Interpretation of test results should be made within the context of the individual patient history and as part of other medical information and testing.

In conclusion and in the spirit of the creative radicalism so long associated with our beautiful surroundings here in Monte Verità, Ascona, Switzerland, I want to look to the future. Such past Monte Verità celebrities as the anarchist, Michail Bakunin, and the dancer, Isadora Duncan, would encourage us to cast off constraining dogma and to move freely among competing viewpoints. We must recognize that nighttime and daytime polysomnographic measures are only electrophysiological epiphenomena that imperfectly reflect underlying CNS processes. As such, polysomnography's ability to address underlying neurobiology has always been limited. Already mentioned is the wide inter-individual variance in outcomes on the MSLT and the MWT in both control and patient populations. This problem is compounded by additional sensitivity issues related to time-of-day effects. Clearly, there is difficulty interpreting results of MSLTs and MWTs run during the usual hours of sleep (e.g., 11:00 PM to 7:00 AM). Most people would appear to be pathologically sleepy during this time period.

There is currently no clinical laboratory test that can identify people who are pathologically sleepy. Looking beyond electrophysiological approaches, one could envision a validated and standardized test kit that can be used on samples of blood, urine, saliva, or even expired gas. Already, assays of cerebrospinal fluid aimed at functional hypocretin transmission are being used in the evaluation of patients with possible narcolepsy and other disorders of excessive somnolence (41–43). It is possible that refinements of this approach could replace nighttime and daytime polysomnography in the evaluation and diagnosis of patients suspected of having narcolepsy. Could one or more molecules be identified as indices of increased sleepiness? A test based on levels of such molecules might identify patients who obtained less, say, than four hours of sleep in, say, the previous 48 hours with sensitivity and specificity equal to or better than nocturnal polysomnography and multiple sleep latency testing. The need for such a biochemical test was identified at least 50 years ago in the context of research on hypnotoxins that are metabolized during wakefulness; detectable in urine, blood or cerebrospinal fluid; and promote sleep after purification and

administration to control animals. Present day femptogram levels of sensitivities in proteomic assays have prompted renewed calls for such tests.

Turning again to present day clinical practice, and recognizing efforts to establish evidenced-based care (11,12), it would seem advisable that any diagnosis of excessive sleepiness be made with as much clinical information as possible. Because such diagnoses can affect earnings and privilege to operate an automobile, the MSL should not be the sole criterion for determining sleepiness or for certifying a diagnosis or response to treatment. Interpretation of test results should be made within the context of the patient's history and testing.

With respect to clinical evaluation for the possible diagnosis of narcolepsy, Aldrich et al., (44) reporting on a series of 2,083 subjects of whom 170 (8.2%) were diagnosed with narcolepsy, concluded that that the MSLT (a) cannot be used in isolation to confirm or exclude narcolepsy, (b) is indicated only in selected patients with excessive daytime sleepiness, and (c) is most valuable when interpreted in conjunction with clinical findings. A more recent report by Sturzenegger and Bassetti (45) also emphasized the importance of integrating clinical findings with electrographic and other ancillary tests and found that specific information on severity of daytime sleepiness and the characteristics of cataplexy suggested the existence of subgroups of narcoleptics as distinct from non-narcoleptic patients with the complaint of sleepiness. The conclusions of the American Academy of Sleep Medicine's Standards of Practice Committee (11,12) are that available evidence does not justify use of a single value for MSLT sleep latency or a single value for number of REM periods observed on the MSLT to rule-out a diagnosis of narcolepsy. It is appropriate for such evaluations to include a nocturnal polysomnogram to rule-out sleep disordered breathing and other conditions not characteristic of narcolepsy that could explain the complaint of excessive sleepiness during the day, followed by an MSLT performed using the protocol detailed in reference 11. Results of these procedures that may be judged concordant with the diagnosis of narcolepsy are as follows:

Nocturnal polysomnogram negative for sleep disordered breathing and so on
MSLT positive for average sleep latency <10 minutes on all naps
MSLT positive for REM on more than one nap

This approach to diagnostic use of nighttime and MSLT polysomnographic data was presaged by the report of Aldrich et al. [Aldrich, 1997 #1788] who found that their highest specificity for narcolepsy was 99.2% with a positive predictive value of 87% using MSLT criteria of three or more episodes of REM sleep combined with an mean sleep latency on the MSLT of <5 minutes, but the sensitivity of this combination was only 46%. When the Aldrich team considered parameters from the nocturnal polysomnogram as diagnostic discriminators, they found that a nocturnal sleep onset REM period combined with a nocturnal sleep latency <10 minutes yielded a specificity of 98.9% and positive predictive value of 73%, but the sensitivity was 27%. Clearly, there is a large body of convergent data pointing to the need to combine all clinical and polysomnographic data in making a diagnosis of narcolepsy. As sleep lab results range away from the above stated ideals, it is prudent to rely more on clinical judgment.

Acknowledgments

Thanks and recognition are due to the MSLT/MWT Taskforce appointed by the American Society for Sleep Medicine: Donna Arand (Chair), Michael Bonnet, Tom Hurwitz, Roger Rosa, and Rahul Sangal. The author also wishes to recognize the key contributions of his collaborators of many years: Roza Hayduk, Milton Erman, and John Harsh. Preparation of this chapter and participation in the symposium was supported by The National Institutes of Neurological Disorders and Stroke (NINDS) of the United States National Institutes of Health (NIH). Mr. William Tucker is recognized for his technical and artistic assistance in manuscript and figure preparation.

References

1. Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy. Volume 3, Advances in Sleep Research. New York: Spectrum Publications; 1976.
2. Dement WC. Daytime sleepiness and sleep "attacks." In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy: Spectrum Publications; 1976:17–42.
3. Vogel G. Studies in psychophysiology of dreams. III. The dream of narcolepsy. Arch Gen Psychiatry 1960; 3:421–428.
4. Rechtschaffen A, Wolpert W, Dement WC, Mitchel S, Fischer C. Nocturnal sleep of narcoleptics. Electroencephalography and Clinical Neurophysiology. 1963; 15:599–609.
5. Takahashi Y, Jimbo M. Polygraphic study of narcoleptic syndrome, with special reference to hypnagogic hallucinations and cataplexy. Folia Psychiatr Neurol Jpn 1964; (suppl 7):343–347.
6. Vogel G. Mentation reported from naps of narcoleptics. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy: Proceedings of the First International Symposium on Narcolepsy, July 1975. New York: Spectrum Publications, Inc.; 1976:161–168.
7. Carskadon MA. The role of sleep-onset REM periods in narcolepsy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy: Proceedings of the First International Symposium on Narcolepsy, July 1975. New York: Spectrum Publications, Inc.; 1976: 499–520.
8. Carskadon MA, Dement WC. The Multiple Sleep Latency Test: What does it measure? Sleep 1982; 5: S67–72.
9. Mitler M, Gujavarty K, Browman CP. Maintenance of wakefulness test: a polysomnographic technique for evaluating treatment in patients with excessive somnolence. Electroencephalogr Clin Neurophysiol 1982; 53:658–661.
10. American Sleep Disorders Association Standards of Practice Committee. Practice parameters for the indications for polysomnography and related procedures. Sleep 1997; 20(6):406–422.
11. Littner MR, Kushida C, Wise M, et al. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. Sleep 2005; 28(1):113–121.
12. Arand D, Bonnet M, Hurwitz T, Mitler M, Rosa R, Sangal RB. The clinical use of the MSLT and MWT. Sleep 2005; 28(1):123–144.
13. Thorpy MT. The clinical use of the Multiple Sleep Latency Test. 1992; 15:268–276.
14. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. Psychophysiol 1973; 10:431–436.
15. Johns MW. A new method for measuring daytime sleepiness: The Epworth Sleepiness Scale. Sleep 1991; 14(6):540–545.
16. Gillberg M, Kecklund G, Akerstedt T. Relations between performance and subjective ratings of sleepiness during a night awake. Sleep 1994; 17(3):236–241.
17. Cook Y, Schmitt F, Berry D, Gilmore R, Phillips B, Lamb D. The effects of nocturnal sleep, sleep disordered breathing and periodic movements of sleep on the objective and subjective assessment of daytime somnolence in healthy aged adults. Sleep Res 1988; 17:95.

18. Sangal RB, Thomas L, Mitler MM. The maintenance of wakefulness test (MWT) and the multiple sleep latency test (MSLT) measure different abilities in patients with sleep disorders. Chest 1992; 101:898–902.
19. US Modafanil in Narcolepsy Multicenter Study Group. Randomized trial of modafanil for the treatment of pathological somnolence in narcolepsy. Ann Neurology 1998; 43:88–97.
20. Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy: US Modafinil in Narcolepsy Multicenter Study Group. Neurology 2000; 54:1166–1175.
21. Harsh J, Peszka J, Hartwig G, Mitler M. Night-time sleep and daytime sleepiness in narcolepsy. J Sleep Res 2000; 9(3):309–316.
22. Doghramji K, Mitler MM, Sangal RB, et al. A normative study of the maintenance of wakefulness test (MWT). Electroencephalogr Clin Neurophysiol 1997; 103:554–562.
23. Carskadon MA, Dement WC. Sleep tendency: an objective measure of sleep loss. Sleep Res 1977; 6:200.
24. Carskadon MA, Dement WC. Effects of total sleep loss on sleep tendency. Percept Mot Skill 1979; 48(2):495–506.
25. Richardson GS, Carskadon MA, Flagg W, Van den Hoed J, Dement WC, Mitler MM. Excessive daytime sleepiness in man: multiple sleep latency measurement in narcoleptic and control subjects. Electroencephalogr Clin Neurophysiol 1978; 45:621–627.
26. Mitler MM, Van den Hoed J, Carskadon MA, et al. REM sleep episodes during the multiple sleep latency test in narcoleptic patients. Electroencephalogr Clin Neurophysiol 1979; 46:479–481.
27. Hartse KM, Zorick F, Sicklesteel J, Piccione P, Roth T. Nap recordings in the diagnosis of daytime somnolence. Sleep Res 1979; 8:190.
28. Van den Hoed J, Kraemer H, Guilleminault C, et al. Disorders of excessive daytime somnolence: polygraphic and clincial data for 100 patients. Sleep 1981; 4:23–37.
29. Dement WC, Carskadon MA, Richardson GS. Excessive daytime sleepiness in the sleep apnea syndrome. In: Guilleminault C, Dement, WE, ed. Sleep Apnea Syndromes. New York: Alan R. Liss; 1978:23–46.
30. Hartse KM, Roth T, Zorick FJ, Zammit G. The effect of instruction upon sleep latency during multiple daytime naps of normal subjects. Sleep Res 1980; 9:123.
31. Dement WC, Seidel WF, Carskadon MA. Daytime altertness, insomnia, and benzodiazepines. Sleep 1982; 5:S28–S45.
32. Carskadon MA, Dement WC, Mitler M, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT): a standard measure of sleepiness. Sleep 1986; 9(4):519–524.
33. Harrison Y, Horne JA. "High sleepability without sleepiness." The ability to fall asleep rapidly without other signs of sleepiness. Neurophysiologie Clinique 1996; 26:15–20.
34. Bonnet MH, Arand DL. 24-Hour metabolic rate in insomniacs and matched normal sleepers. Sleep 1995; 18:581–588.
35. Haynes SN, Fitzgerald SG, Shute GE, Hall M. The utility and validity of daytime naps in the assessment of sleep-onset insomnia. J Behav Med 1985; 8(3):237–247.
36. Stepanski E, Zorick F, Roehrs T, Young D, Roth T. Daytime alertness in patients with chronic insomnia compared with asymptomatic control subjects. Sleep 1988; 11(1):54–60.
37. Bonnet MH, Arand DL. Activity, arousal, and the MSLT in patients with insomnia. Sleep 2000; 23: 205–212.
38. Mitler MM, Doghramji K, Shapiro C. The maintenance of wakefulness test normative data by age. J Psychosomatic Research 2000; 49:363–365.
39. Mitler MM, Gujavarty KS, Sampson MG, Browman CP. Multiple daytime nap approaches to the sleepy patient. Sleep 1982; 5:S119–S127.
40. Johns MW. Sensitivity and specificity of the multiple sleep latency test (MSLT), the maintenance of wakefulness test and the Epworth sleepiness scale: Failure of the MSLT as a gold standard. J Sleep Res 2000; 9:5–11.
41. Ripley B, Overeem S, Fujiki N, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001; 57 (12): 2253–2258.
42. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59 (10): 1553–1562.

43. Ebrahim IO, Sharief MK, de Lacy S, et al. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurosurg Psychiatry 2003; 74(1):127–130.
44. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 1997; 20(8):620–629.
45. Sturzenegger C, Bassetti CL. The clinical spectrum of narcolepsy with cataplexy: a reappraisal. J Sleep Res 2004; 13(4):395–406.

23
Canine Narcolepsy: History and Pathophysiology

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Early History

Narcolepsy research during the last 50 years has been greatly facilitated by the existence of a unique animal model, canine narcolepsy (Table 1). The existence of this model was first suspected in 1972 when Dr. William Dement presented recordings of human cataplectic attacks during a convention of the American Medical Association (San Francisco) and a neurologist in attendance attested to the existence of a dog with similar symptoms. The affected canine, unfortunately, already had been sacrificed, as was customary at this time for diseased animals. A Poodle with similar symptoms, "Monique," was fortunately soon identified in Saskatoon, Canada, and donated to Stanford University, where a colony of narcoleptic canines was formally established.

The first publications on canine narcolepsy quickly followed, with the almost simultaneous reports of Knecht et al. (1) and Mitler et al. (2). The report of Knecht et al. also mentions a feline case, though the described episodes are more likely to be epileptic seizures than narcolepsy; in fact, it is noteworthy to mention that narcolepsy has never been reported in cats, in spite of a large pet population and a profound atonia during rapid eye movement (REM) sleep in this species. Rather, narcolepsy has been commonly reported in horses, either as a sporadic disease or as a genetic disorder (Shetland ponies), and more rarely and less convincingly in sheep, bulls, and donkeys. In goats, narcolepsy is also frequently confused with myotonia congenita (a channelopathy due to a mutation of the skeletal muscle voltage-dependent chloride channel), the so-called "fainting goats." Since these initial reports, narcolepsy has been identified in more than 20 canine breeds in multiple countries including United States, South Africa and Japan (Table 2). In almost all cases, the disease is sporadic and occurs with a variable age of onset ranging from seven weeks to seven years (3). Early breeding experiments in Poodles and Beagles, including backcrosses, were unsuccessful in generating genetic transmission (4). In 1975, however, a Doberman family with multiple affected offspring was identified and a similar multiplex family was identified in Labrador Retrievers. Autosomal recessive genetic transmission was demonstrated in both breeds, without complementation, indicating a single locus (4). In Dobermans and Labradors, in contrast to non-genetic cases, onset is often as early as a few weeks of age (mean onset 8.2 and 14.4 weeks respectively), and disease severity typically peaks around six months of age and may decrease with advancing age.

Table 1 An Historical Account of Canine Narcolepsy

Year	Historical account	References
1972	The existence of "canine narcolepsy" is suspected after a presentation at the American Medical Association	
1973–1974	First published reports of canine narcolepsy	1,2
1975	Identification of a Doberman pedigree with multiple affected members	3,4
1975–1982	Genetic transmission in Labradors and Dobermans, and absence of simple transmission in Poodles and Beagles	3,4
1977–1979	Sleep recordings and MSLT-like testing in sporadic cases confirm daytime sleepiness and cataplexy	6–9
1983–1997	Sleep recordings and MSLT-like testing in familial cases confirm daytime sleepiness and cataplexy	9–11
1985	Neurochemical and pharmacological studies suggest cholinergic/monoaminergic imbalance in narcolepsy	13,14
1987	Dog leukocyte antigen studies in sporadic and familial cases indicate no DR and DQ assocation in canine narcolepsy	42,43
1988	Doberman animals are transferred to University of California Los Angeles to establish a viable satellite breeding colony	
1989–1999	Backcrosses and linkage analysis using minisatellite markers and candidate gene probes in canine narcolepsy	
1991	Indentification of a linkage marker for canine narcolepsy, a DNA segment with high homology with the immunoglobulin μ-switch region	41
1993	Adrenergic but not dopamine or serotonin reuptake inhibition as the mode of action of antidepressant's effect on canine cataplexy	24,25
1994	Cloning, mapping and sequencing of the immunoglobulin-like marker as an anonymous GC rich, repetitive DNA segment without function	44
1993–1998	The wake promoting effects of amphetamine-stimulants and modafinil are likely mediated by presynaptic effects on dopamine reuptake and/or release	26,27
1997	Building of a Bacterial Artificial Chromosome (BAC) genomic library using a canine heterozygote animal	45
1997	Canine chromosomal Fluorescence In situ Hybdridization (FISH) and systemic mapping studies between human chromosome 6 and canine chromosome 12	46
Spring 1999	Identification of a genomic insertion in the vicinity of the hypocretin receptor 2 (HCRTR2) locus	46
1999	Sequencing of two exon-skipping HCRTR2 mutations causing canine narcolepsy in Labradors and Dobermans	46
1999–2004	During cataplexy the activity of REM off cells of the adrenergic locus coeruleus, serotoninergic raphe magnus and histaminergic tuberommillary nucleus are, respectively, low, intermediary and high	52–54
2000	Sequencing of DLA-DQB1 in sporadic and familial cases indicates no DLA-DQ association	49
2001	Identification and functional characterization of a single amino acid substitution in HCRTR2 causing narcolepsy in a Daschund multiplex family	47
2002	Low to undetectable CSF and brain hypocretin peptide concentration is found in spoardic but not familial canine narcolepsy	48

Table 2 Canine Breeds Affected with Narcolepsy

Breeds	Sporadic cases[a]	Familial cases[a]	Pathophysiology, if established	References
Poodle (standard and miniature)	10		Hypocretin deficiency (low CSF and brain hypocretin-1 [Hcrt-1], n = 1); lack of genetic transmission established by colony breeding	2,4,5,48
Dachshund (7 US, 1 South Africa)	7	1	In a sporadic case, hypocretin deficiency (low CSF Hcrt1)	1,4,5,48
			In a single multiplex family, hypocretin receptor 2 (HCRTR2) exon1 mutation (G461A, E54K), with loss of hcrt-1 ligand binding	45
Beagle	3		Lack of genetic transmission established by colony breeding	4
Doberman pinscher	1	4[b]	In familial cases, HCRTR2 exon skipping mutation secondary to the insertion of a large SINE repeat in the Lariat sequence area upstream of coding exon 4; leads to a non functional truncated protein	3,4,46,47
Irish setter	2			4
St. Bernard	2			4
Labrador	1	1[b]	In familial cases, HCRTR2 "exon" skipping mutation secondary to a single nucleotide substitution in the intron-exon boundary of coding exon 6; leading to a non functional truncated protein	3,4,46,47
Australian shepherd	1			4
Wire haired griffon	1			4
Cocker spaniel	1			4
Springer spaniel	1			4
Afghan	1			4
Airdale	1			4
Malamute	1			4
Welsh corgi	1			4
Weimaraner	1			51
Belgian skiperkee	1		Hypocretin deficiency (low CSF Hcrt-1)	50
Chihuahua (Japan)	1		Hypocretin deficiency (low CSF Hcrt-1)	55
Mixed breeds[c]	3		Hypocretin deficiency (low brain Hcrt-1 in a Cocker poodle)	4,48

[a] Individual animals not known to be related, in some cases, however, the same mutation was found indicating identity by descent.
[b] Also established by colony breeding experiments.
[c] 1 Chihuahua-terrier, 2 Cocker poodles. Animals are from the United States, unless specified.

II. Early Clinical, Pharmacological and Electrophysiological Characterization of the Canine Narcolepsy Model

Early experiments were first focused on establishing the canine model as similar to human narcolepsy. The first experiments were performed in sporadic canines with narcolepsy. As mentioned above, sporadic cases have typically later and more variable disease onset than familial cases, a picture more consistent with human narcolepsy. Severity for cataplexy is generally more severe than in humans, with hundreds of attacks often observed every day. Whether cataplexy severity in sporadic cases is the result of an inclusion bias (severely affected dogs were more likely to be identified and donated) or reflects genuine differences in the expression of the phenotype across species is unknown. Muscle weakness (cataplexy) could be induced in these dogs by sudden emotion, such as the presentation of good food or playing (Figure 1). This led to the establishment of a behavioral test to quantify cataplexy, the Food Elicited Cataplexy Test (FECT). Like human cataplexy and unlike epilepsy,

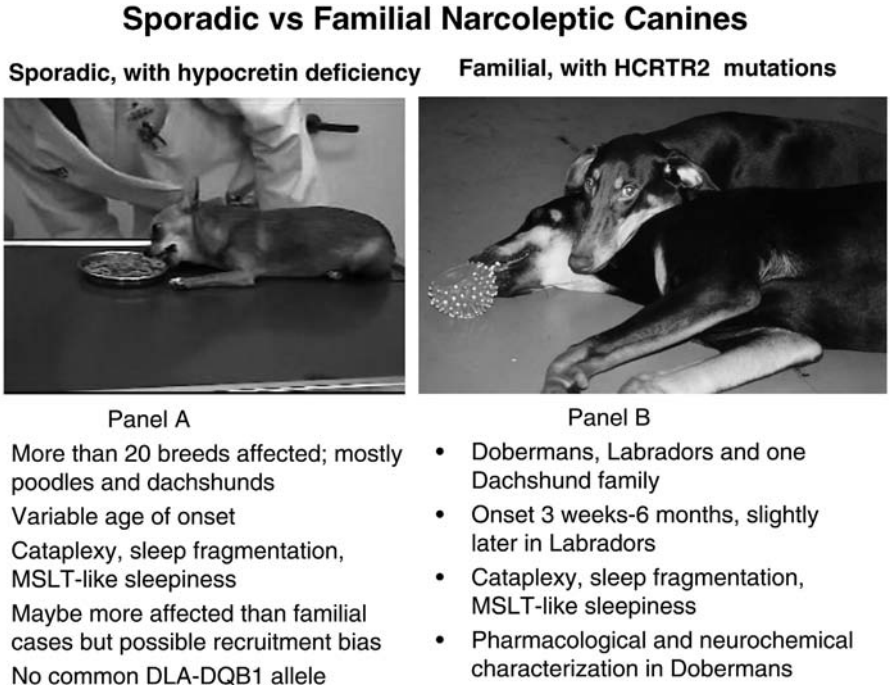

Sporadic vs Familial Narcoleptic Canines
Sporadic, with hypocretin deficiency
Familial, with HCRTR2 mutations
Panel A
More than 20 breeds affected; mostly poodles and dachshunds
Variable age of onset
Cataplexy, sleep fragmentation, MSLT-like sleepiness
Maybe more affected than familial cases but possible recruitment bias
No common DLA-DQB1 allele
Panel B
Dobermans, Labradors and one Dachshund family
Onset 3 weeks-6 months, slightly later in Labradors
Cataplexy, sleep fragmentation, MSLT-like sleepiness
Pharmacological and neurochemical characterization in Dobermans

Figure 1 Narcoleptic Dobermans (HCRTR2 mutated, panel B) and a narcoleptic chihuahua (hypocretin deficient, panel A) in the midst of a cataplectic attack. Note the muscle paralysis and open eyes. Cataplectic attacks in dogs are typically triggered by the excitement of play (panel B) or the presentation of appetizing food (panel A). The presentation of 10–12 pieces of food on the floor while recording the number of elicited cataplectic attacks and time elapsed to complete the test is used to test for the severity of cataplexy, a procedure called the Food Elicited Cataplexy Test (FECT). *Source:* Courtesy of Drs. Nishino (panel B) and Tonokura (panel A).

canine cataplexy was found to be highly responsive to imipramine (5). As with REM sleep, and unlike muscle weakness in myasthenia gravis, cataplexy in dogs was exacerbated by physostigmine, an acetylcholinesterase inhibitor (5). EEG/EMG recordings during cataplexy indicated muscle atonia without associated theta activity (as opposed to during REM sleep that has muscle atonia with theta activity) (5). Twenty-four hour EEG recording studies in Poodles and Beagles reported a large range of cataplexy severity and close to normal amounts of sleep over 24 hours, but with increased amounts of drowsy-like EEG stages and an overall sleep/wake fragmentation (6,7). A Multiple Sleep Latency Test (MSLT)-like procedure was also established where the time to sleep onset was measured in the dark under an alternating 30 minutes of light (wake) and 30 minutes of darkness (sleep permitted). Using this test, daytime sleep latencies were found to be dramatically shorter (1–4 minutes) in narcoleptic Poodles ($n = 2$) and a Beagle, as compared to control dogs (10–28 minutes) (8).

Due to the difficulties in obtaining a regular supply of animals with sporadic narcolepsy, and appropriate breed-matched controls, further experiments were performed in dogs with the genetic form of narcolepsy, a subset of Dobermans and Labradors. The phenotype in the genetic form of canine narcolepsy was noted to be similar, but generally less severe than that observed in sporadic canine narcolepsy, a difference that may be due to a difference in pathophysiology (now known to be hypocretin deficiency versus hypocretin receptor-2 (HCRTR2) mutation) and/or the fact sporadic cases were donated to the colony and thus more likely to be more severely affected (3,4). EEG studies during cataplexy indicated a wake-like EEG pattern with atonia that was followed by a REM-like EEG when the attacks were pronounced (9). Twenty-four hour recording studies also found sleep fragmentation and increased drowsy/light sleep, as was reported in sporadic cases (10,11). Evaluation of the suprachiasmatic nucleus and the 24 hours cerebro spinal fluid (CSF) melatonin rhythm was also performed and essential timekeeping function was found to be normal (12). Finally, more recently, we also found that daytime MSLT-like studies in this model indicate reduced sleep latency to drowsy, light and REM sleep (13). The specific occurrence of sleep onset REM periods (SOREMPs), as defined by REM occurrence within 15 minutes of sleep onset, was also observed in narcoleptic, but not control Dobermans (13).

Similar to sporadic cases, cataplexy was found to be stimulated by centrally acting cholinergic agonists (acetylcholinesterase inhibitors and muscarinic agonists) and decreased by monoaminergic reuptake inhibitors (tricyclic antidepressants, fluoxetine, and nisoxetine), suggesting a cholinergic-monoaminergic imbalance (14).

III. Neurochemical Studies in Canine Narcolepsy

In collaboration with Drs. Jack Barchas and Roland Ciaranello at Stanford University, a series of neurochemical studies were performed primarily using the Doberman model. These experiments included monoamine and metabolite measurements in the CSF and brain tissue, and radioreceptor binding and autoradiography studies. CSF dopamine (DA), serotonin (5-HT) and norepinephrine (NE) metabolite studies in human and canine narcolepsy yielded variable results (14). A systematic study of DA, 5-HT, NE and their metabolites was performed in 150 brain regions (15), and further replicated

(16). The most consistent findings were that DA and DOPAC increase in the amygdala and, to a lesser extent, in other brain regions. NE was also found to be elevated in the preoptic hypothalamus (16), while serotoninergic indices were generally normal. These findings were generally interpreted as reflecting decreased DA turnover, as the DOPAC/DA ratio was decreased and a decreased monoaminergic tone was a more logical abnormality to explain narcolepsy. Interestingly, recent studies indicate not only increased DA/NE content in canine narcolepsy but also decreased histamine concentration in brain tissue from narcoleptic dogs with a mutated HCRTR2 (17), suggesting differential compensatory mechanisms for various monoamines.

Receptor binding studies were also initiated. Benzodiazepine receptors were found to be normally distributed (18). In contrast, an upregulation of DA D2 receptors was noted in the nucleus accumbens, rostral caudate, and amygdala, suggesting receptor upregulation secondary to decreased DA turnover (19). A consistent and reproducible increase in muscarinic M2 receptors was noted in the pontine reticular formation (PRF), a region where muscarinic stimulation produces REM sleep and atonia (20,21). Changes in locus coeruleus alpha-2 adrenergic receptors were also found (22).

IV. Further Pharmacological Studies in Canine Narcolepsy

In 1986, modafinil, a compound believed at the time to promote wakefulness by stimulation of alpha-1 receptors, was studied in canine narcolepsy by the author. A series of alpha-1 adrenergic drugs was also studied (23). Interestingly, modafinil was found to subjectively increase wakefulness, but did not have any effects on cataplexy in either sporadic or genetically determined dogs at doses up to 80 mg/kg per os. In contrast, alpha-1 compounds, most notably those acting on the alpha-1b subtype, were found to strongly modulate cataplexy, suggesting the importance of adrenergic mechanisms in the control of cataplexy rather than sleepiness (23). Together with the late Dr. Alain Renaud, we studied the effects of various selective adrenergic, dopaminergic, and serotonergic reuptake inhibitors and found adrenergic reuptake inhibition to be critical to the mode of action of antidepressants on cataplexy (24). Parallel experiments with Dr. Seiji Nishino, who joined our group in 1987, indicated that the activity of many antidepressants on cataplexy was mediated by active metabolites with higher affinity for the adrenergic reuptake site (25). Dr. Nishino's major interest was initially in the area of prostaglandin research, but he quickly became interested in extending pharmacological studies beyond this area of research.

The effects of selective DA reuptake inhibitors on subjective alertness without any effect on cataplexy (24) led us to reconsider modafinil as a potential DA reuptake inhibitor (26). Modafinil was found to bind the DA transporter (DAT) with low affinity, and experiments with Dr. Nishino (27) found excellent correlations between EEG induced wakefulness and DAT binding profiles for various DAT inhibitors. Later, elegant experiments by Dr. Takashi Kanbayashi also found that various amphetamine derivatives and enantiomers increase wakefulness by stimulation of DA release, while the anticataplectic effects of these stimulants at high doses is mediated through their additional effects on adrenergic transmission (28).

In parallel with these studies, we studied the effects of various selective receptor ligands on sleep and cataplexy in narcoleptic canines (13). To ensure pharmacologic

specificity, all studies were conducted using a series of structurally diverse chemical entities sharing a specific mode of action. Surprisingly, whereas DA reuptake inhibitors increased wakefulness without affecting REM sleep or cataplexy (27), D2/D3 agonists and antagonists were found to increase and decrease cataplexy, respectively (29). The differential effects on wakefulness versus cataplexy of DAT inhibitors versus D2/D3 compounds remains to be explained. The fact that most D2/D3 agonists and L-DOPA are not strongly wake-promoting in clinical practice is generally explained by a preferential presynaptic effect of these compounds at low dose, an effect that paradoxically reduces DA transmission in some projection areas. Indeed, DA agonists/antagonists typically have biphasic effects on locomotion, with only high doses of DA agonists causing an increase in locomotion that is often accompanied by stereotypies. A differential sensitivity of various DA cell groups or terminals to DA reuptake inhibition, or presynaptic versus postsynaptic DA receptor modulation may thus be responsible for the striking differential effects of DA reuptake blockers and agonists on motor related symptoms versus sleep/wake. These differential neuroanatomical effects may also explain the observation that selective DAT inhibitors, in contrast to D2/D3 agonists or antagonists, do not modulate cataplexy when administered to narcoleptic canines.

Other effects of interest included a 5-HT1a modulation of cataplexy (30), thyrotropin-releasing hormone effects on sleepiness and cataplexy (31) and a histamine H3 receptor-mediated reduction of cataplexy and sleepiness (17). These experiments illustrate the complexity of the neuronal network and receptor inputs regulating sleepiness and cataplexy after systemic administration.

V. Local Injections and In Vivo Dialysis Studies

The difficulties in interpreting the effects of systemic drug administration, together with observation of M2 and other receptor abnormalities led us to study the functional significance of these abnormalities through local injections and in vivo microdialysis (32–37). These experiments, carried with Drs. Nishino and Malcolm Reid, indicated hypersensitivity to M2 cholinergic stimulation not only in the PRF (32,33), but also in the basal forebrain area, where muscarinic M2/M3 stimulation was able to produce cataplexy only in narcoleptic animals (34,35). Acetylcholine release was also increased in the PRF during cataplexy (33), paralleling findings reported during REM sleep. Interestingly, it was also found that acetylcholine release in the basal forebrain was increased by FECTs in both control and narcoleptic animals (35), suggesting that increased release on hypersensitive receptors in this area could be a trigger for cataplexy (35). We also found that D2/D3 autoreceptor stimulation in the brainstem modulated cataplexy and, to a lesser extent, drowsy sleep, suggesting that the D2/D3 effects on cataplexy are indeed modulated by presynaptic effects on dopaminergic transmission (36,37). Interestingly, we were unable to find the site of action of adrenergic modulation through local or intracerebroventricular injections of alpha-1 or adrenergic reuptake inhibitors, an observation that may suggest modulation of cataplexy and muscle tone through established adrenergic projections to the spinal cord and alpha-1b modulation of motoneurons. Muscle tone modulation through alpha-1 receptors on motoneurons has been suggested by others (38,39).

VI. Positional Cloning Studies in Canine Narcolepsy

Pharmacological and neurochemical experiments using narcoleptic canines substantiated secondary monoaminergic (dopaminergic, noradrenergic) and cholinergic abnormalities. More recently, decreased histamine transmission in both sporadic and familial cases has been reported (17). A monoaminergic-cholinergic imbalance was also illustrated by the finding of dramatic increases in cataplexy when indirect cholinergic agonists and monoaminergic depressors were coadministrated (40). In some cases, cataplexy could even be induced in heterozygous, asymptomatic animals (40). These experiments were critical to the design of our future genetic linkage studies, where backcross animals were raised to study the occurrence of spontaneous cataplexy, then challenged by cataplexy-enhancing drugs to confirm phenotype status in all cases.

Genetic linkage and Doberman backcrossing studies were initiated with Dr. Carl Grumet's help in 1989. In the absence of a genetic map in dogs, minisatellite probes and candidate gene probes (restriction fragment length polymorphisms, RFLPs) were used to search for genetic markers (41). Tight linkage with the dog leukocyte antigen (DLA) was excluded (41), in agreement with earlier studies indicating no DLA association in familial and sporadic cases of canine narcolepsy (42,43). Curiously, however, weak but non-significant linkage within 30 cM of DLA was noted, a finding that we initially dismissed considering the tight HLA association in humans (41). As canarc-1 (the original name of the canine narcolepsy gene that is now known to be the hypocretin receptor 2 or HCRTR2) and DLA are in fact on the same canine (chromosome 12) and human chromosome (chromosome 6), this finding was later found to be correct. A linkage marker was also quickly found using a GC-rich repetitive probe for the human μ-switch immunoglobulin segment (a recombination signal segment for immunoglobulin class switching). This probe generated several distinct polymorphic bands including a main locus not linked with canarc-1 and weaker cross-reacting bands tightly linked with canine narcolepsy (41).

Further genetic studies were greatly slowed by the lack of genomic and genetic resources available in canines. The cloning of the μ-switch-like linkage marker only yielded a repetitive sequence and no associated immunoglobulin chain constant gene (44). Together with Dr. Peter De Yong, we constructed our own large insert genomic Bacterial Artificial Chromosome (BAC) library using Grumpy, a dog heterozygous for narcolepsy (45). The library was first used to chromosome walk around our initial μ-switch-like marker. Sequencing the end of a BAC clone, we discovered an exon for a human Myo6, a gene known to localize onto human chromosome 6. With Dr. Juliette Faraco, we also did chromosomal fluorescence in situ hybridization (FISH) in canines and found our gene to be localized on canine chromosome 12 in a large region of synteny with human chromosome 6 (46).

Our BAC library was next screened with human Expressed Sequence Tag (EST) probes known to locate within the region of interest in humans. This allowed us to isolate multiple canine genomic contigs and to complete the physical isolation of the region by chromosome walking starting simultaneously from these areas. Chromosome walking, microsatellite marker isolation, development and testing in backcrosses proceeded from these areas, with much of the work being performed by Dr. Ling Lin. In 1999, a small genomic segment containing only two known genes was identified as the segment of interest. Using a probe for one of these two genes, hypocretin receptor

2, we found major RFLPs between disease-associated and control BAC haplotypes suggesting a genomic rearrangement close to HCRTR2 in narcoleptic Dobermans. BAC genomic sequencing studies in the region, together with reverse transcription (RT)-PCR of expressed HCRTR2 mRNA indicated that a large SINE repeat was inserted upstream of the coding exon 4, leading to exon 4 skipping and the generation of a truncated transcript with a premature stop codon. A distinct coding exon 6 skipping mutation was also found in narcoleptic Labrador Retrievers (46), while a single amino acid substitution in the extracellular N-terminal end of the receptor with loss of hypocretin binding was observed in a Dachshund family (47). All identified mutations were found to result in a complete loss of function of HCRTR2 (47).

VII. Hypocretin Deficiency Without DLA-DQB1 Association in Sporadic Narcolepsy Cases

The finding of HCRTR2 mutations led us to evaluate hypocretin transmission in human narcolepsy and sporadic canine cases. Mutations were not discovered in either the hypocretin locus or the HCRTR2 locus in sporadic canine cases (47). Rather, a loss of hypocretin-1 in the CSF and a loss of hypocretin peptide brain content was found (48), as was found in human narcolepsy. This finding led us to sequence dog leukocyte antigen (DLA) DQ genes in a large number of sporadic narcoleptic dogs (49). Surprisingly, unlike human narcolepsy, we could not find any significant DQB1 allele sharing in sporadic, hypocretin-deficient narcoleptic canines.

VIII. Perspectives

The discovery of hypocretin deficiency in human narcolepsy has led to the establishment of rodent models for the disorder, as discussed elsewhere in this volume. These models have narcolepsy, are significantly cheaper, and are less controversial to maintain than are canines. A disadvantage is the difficulty in distinguishing cataplexy with SOREMPs, as the emotional trigger for cataplectic episodes is not obvious in rodents. Most problematically, hypocretin supplementation may be one of the most interesting areas for future therapeutic investigation and cannot be investigated using the genetic form of canine narcolepsy, a receptor deficient form of narcolepsy. To illustrate this further, we recently found that intracerebroventricular injections of high doses of hypocretin-1, which are profoundly wake-promoting in control dogs, do not affect sleepiness or cataplexy in HCRTR2 mutated canines (50).

Our opinion is that only the sporadic form of canine narcolepsy with hypocretin deficiency remains a useful model for future studies. The lack of DLA-DQB1 association in sporadic narcolepsy raises the interesting possibility of using this model to discover other potentially involved HLA or non-HLA genetic factors; this could lead to understanding the cause of hypocretin cell loss in human narcolepsy.

An additional use for canine narcolepsy may be in the therapeutic arena, as sporadic canine narcolepsy is clinically close to human narcolepsy. Unfortunately, however, precisely because of the sporadic nature of the disease, these animals are difficult to acquire in large numbers. We have recently received a donation of one such dog (50), with the understanding he could only be used for in vivo pharmacology, and

have shown that large doses of intravenous hypocretin-1 in this dog only transitorily reduced cataplexy (50). It is our experience that attitudes have changed and that pet owners or breeders are no longer willing to donate affected dogs for research. Rather, they are interested in using the large amount of knowledge we have accumulated (13) and our expertise to treat these animals. We recently attempted to rescue the phenotype of narcolepsy in a 3 year-old Weimaraner by perfusing hypocretin-1 into the cisterna magna using a time-release Medtronic pump (51). It was our hope that hypocretin-1 would backflow through the foramen ovale into the cerebral ventricular system, and that a similar device could be used after in the humans with catheterization of the lumbar sac (51). These experiments were unsuccessful, suggesting the impracticality of this approach, but illustrate the use of the canine model for clinical experiments prior to attempting similar human trials. This may be especially useful when or if hypocretin agonists become available.

References

1. Knecht CD, Oliver JE, Redding R, Selcer R, Johnson G. Narcolepsy in a dog and a cat. J Am Vet Med Assoc 1973; 162(12):1052–1053.
2. Mitler MM, Boysen BG, Campbell L, Dement WC. Narcolepsy-cataplexy in a female dog. Exp Neurol 1974; 45(2):332–340.
3. Foutz AS, Mitler MM, Cavalli-Sforza LL, Dement WC. Genetic factors in canine narcolepsy. Sleep 1979; 1(4):413–421.
4. Baker TL, Foutz AS, McNerney V, Mitler MM, Dement WC. Canine model of narcolepsy: genetic and developmental determinants. Exp Neurol 1982; 75(3):729–742.
5. Mitler MM, Soave O, Dement WC. Narcolepsy in seven dogs. J Am Vet Med Assoc 1976; 168(11):1036–1038.
6. Mitler MM, Dement WC. Sleep studies on canine narcolepsy: pattern and cycle comparisons between affected and normal dogs. Electroencephalogr Clin Neurophysiol 1977; 43(5):691–699.
7. Lucas EA, Foutz AS, Dement WC, Mitler MM. Sleep cycle organization in narcoleptic and normal dogs. Physiol Behav 1979; 23(4):737–743.
8. Lucas EA, Foutz AS, Mitler MM, Brown D, Dement WC. Multiple Sleep Latency test in normal and narcoleptic dogs. Soc Neurosci Abstracts 1978; 6:541.
9. Kushida CA, Baker TL, Dement WC. Electroencephalographic correlates of cataplectic attacks in narcoleptic canines. Electroencephalogr Clin Neurophysiol 1985; 61(1):61–70.
10. Kaitin KI, Kilduff TS, Dement WC. Evidence for excessive sleepiness in canine narcoleptics. Electroencephalogr Clin Neurophysiol 1986; 64(5):447–454.
11. Kaitin KI, Kilduff TS, Dement WC. Sleep fragmentation in canine narcolepsy. Sleep 1986; 9(1 Pt 2):116–119.
12. Schwartz WJ, Morton MT, Williams RS, Tamarkin L, Baker TL, Dement WC. Circadian timekeeping in narcoleptic dogs. Sleep 1986; 9(1 Pt 2):120–125.
13. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol 1997; 52(1):27–78.
14. Baker TL, Dement WC. Canine narcolepsy-cataplexy syndrome: evidence for an inherited monoaminergic-cholinergic imbalance. In: Brain Mechanisms of Sleep, McGinty DJ et al. eds. New York:Raven Press 1985; 1999–2044.
15. Mefford IN, Baker TL, Boehme R, Foutz AS, Ciaranello RD, Barchas JD, Dement WC. Narcolepsy: biogenic amine deficits in an animal model. Science 1983; 220(4597):629–632.
16. Miller JD, Faull KF, Bowersox SS, Dement WC. CNS monoamines and their metabolites in canine narcolepsy: a replication study. Brain Res 1990; 509(1):169–171.
17. Nishino S, Fujiki N, Ripley B, Sakurai E, Kato M, Watanabe T, Mignot E, Yanai K. Decreased brain histamine content in hypocretin/orexin receptor-2 mutated narcoleptic dogs. Neurosci Lett 2001; 313(3):125–128.

18. Bowersox SS, Kilduff TS, Kaitin KI, Dement WC, Ciaranello RD. Brain benzodiazepine receptor characteristics in canine narcolepsy. Sleep 1986; 9(1 Pt 2):111–115.
19. Bowersox SS, Kilduff TS, Faull KF, Zeller-DeAmicis L, Dement WC, Ciaranello RD. Brain dopamine receptor levels elevated in canine narcolepsy. Brain Res 1987; 402(1):444–448.
20. Boehme RE, Baker TL, Mefford IN, Barchas JD, Dement WC, Ciaranello RD Narcolepsy: cholinergic receptor changes in an animal model. Life Sci 1984; 34(19):1825–1828.
21. Kilduff TS, Bowersox SS, Kaitin KI, Baker TL, Ciaranello RD, Dement WC. Muscarinic cholinergic receptors and the canine model of narcolepsy. Sleep 1986; 9(1 Pt 2):102–106.
22. Fruhstorfer B, Mignot E, Bowersox S, Nishino S, Dement WC, Guilleminault C. Canine narcolepsy is associated with an elevated number of alpha 2-receptors in the locus coeruleus. Brain Res 1989; 500(1–2):209–214.
23. Mignot E, Guilleminault C, Bowersox S, Frusthofer B, Nishino S, Maddaluno J, Ciaranello R, Dement WC. Central alpha 1 adrenoceptor subtypes in narcolepsy-cataplexy: a disorder of REM sleep. Brain Res 1989; 490(1):186–191.
24. Mignot E, Renaud A, Nishino S, Arrigoni J, Guilleminault C, Dement WC. Canine cataplexy is preferentially controlled by adrenergic mechanisms: evidence using monoamine selective uptake inhibitors and release enhancers. Psychopharmacol (Berl) 1993; 113(1):76–82.
25. Nishino S, Arrigoni J, Shelton J, Dement WC, Mignot E. Desmethyl metabolites of serotonergic uptake inhibitors are more potent for suppressing canine cataplexy than their parent compounds. Sleep 1993; 16(8):706–712.
26. Mignot E, Nishino S, Guilleminault C, Dement WC. Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep 1994; 17(5):436–437.
27. Nishino S, Mao J, Sampathkumaran R, Shelton J, Mignot E. Increased dopaminergic transmission mediates the wake-promoting effects of CNS stimulants. Sleep Res Online 1998; 1:49–61.
28. Kanbayashi T, Honda K, Kodama T, Mignot E, Nishino S. Implication of dopaminergic mechanisms in the wake-promoting effects of amphetamine: a study of D- and L-derivatives in canine narcolepsy. Neurosci 2000; 99(4):651–659.
29. Nishino S, Arrigoni J, Valtier D, Miller JD, Guilleminault C, Dement WC, Mignot E. Dopamine D2 mechanisms in canine narcolepsy. J Neurosci 1991; 11(9):2666–2671.
30. Nishino S, Shelton J, Renaud A, Dement WC, Mignot E. Effect of 5-HT1A receptor agonists and antagonists on canine cataplexy. J Pharmacol Exp Ther 1995; 272(3):1170–1175.
31. Nishino S, Arrigoni J, Shelton J, Kanbayashi T, Dement WC, Mignot E. Effects of thyrotropin-releasing hormone and its analogs on daytime sleepiness and cataplexy in canine narcolepsy. J Neurosci 1997; 17(16):6401–6408.
32. Reid MS, Tafti M, Nishino S, Siegel JM, Dement WC, Mignot E. Cholinergic regulation of cataplexy in canine narcolepsy in the pontine reticular formation is mediated by M2 muscarinic receptors. Sleep 1994; 17(5):424–433.
33. Reid MS, Siegel JM, Dement WC, Mignot E. Cholinergic mechanisms in canine narcolepsy–II. Acetylcholine release in the pontine reticular formation is enhanced during cataplexy. Neurosci 1994; 59(3):523–530.
34. Nishino S, Tafti M, Reid MS, Shelton J, Siegel JM, Dement WC, Mignot E. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. J Neurosci 1995; 15(7 Pt 1):4806–4814.
35. Reid MS, Nishino S, Tafti M, Siegel JM, Dement WC, Mignot E. Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. Exp Neurol 1998; 151(1):89–104.
36. Reid MS, Tafti M, Nishino S, Sampathkumaran R, Siegel JM, Mignot E. Local administration of dopaminergic drugs into the ventral tegmental area modulates cataplexy in the narcoleptic canine. Brain Res 1996; 733(1):83–100.
37. Okura M, Fujiki N, Kita I, Honda K, Yoshida Y, Mignot E, Nishino S. The roles of midbrain and diencephalic dopamine cell groups in the regulation of cataplexy in narcoleptic Dobermans. Neurobiol Dis 2004; 16(1):274–282.
38. Menon MK, Kodama CK, Kling AS, Fitten J. An in vivo pharmacological method for the quantitative evaluation of the central effects of alpha 1 adrenoceptor agonists and antagonists. Neuropharmacology 1986; 25(5):503–8.
39. Volgin DV, Mackiewicz M, Kubin L. Alpha(1B) receptors are the main postsynaptic mediators of adrenergic excitation in brainstem motoneurons, a single-cell RT-PCR study. J Chem Neuroanat 2001; 22(3):157–66.

40. Mignot E, Nishino S, Sharp LH, Arrigoni J, Siegel JM, Reid MS, Edgar DM, Ciaranello RD, Dement WC. Heterozygosity at the canarc-1 locus can confer susceptibility for narcolepsy: induction of cataplexy in heterozygous asymptomatic dogs after administration of a combination of drugs acting on monoaminergic and cholinergic systems. J Neurosci 1993; 13(3):1057–1064.
41. Mignot E, Wang C, Rattazzi C, Gaiser C, Lovett M, Guilleminault C, Dement WC, Grumet FC. Genetic linkage of autosomal recessive canine narcolepsy with a mu immunoglobulin heavy-chain switch-like segment. Proc Natl Acad Sci U S A 1991; 15; 88(8):3475–3478.
42. Dean RR, Kilduff TS, Dement WC, Grumet FC. Narcolepsy without unique MHC class II antigen association: studies in the canine model. Hum Immunol 1989; 25(1):27–33
43. Motoyama M, Kilduff TS, Lee BS, Dement WC, McDevitt HO. Restriction fragment length polymorphism in canine narcolepsy. Immunogenetics 1989; 29(2):124–126.
44. Mignot E, Bell RA, Rattazzi C, Lovett M, Grumet FC, Dement WC. An immunoglobulin switchlike sequence is linked with canine narcolepsy. Sleep 1994; 17(8 Suppl):S68-S76.
45. Li R, Mignot E, Faraco J, Kadotani H, Cantanese J, Zhao B, Lin X, Hinton L, Ostrander EA, Patterson DF, de Jong PJ. Construction and characterization of an eightfold redundant dog genomic bacterial artificial chromosome library. Genomics 1999; 58(1):9–17.
46. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98(3):365–376.
47. Hungs M, Fan J, Lin L, Lin X, Maki RA, Mignot E. Identification and functional analysis of mutations in the hypocretin (orexin) genes of narcoleptic canines. Genome Res 2001; 11(4):531–539.
48. Ripley B, Fujiki N, Okura M, Mignot E, Nishino S. Hypocretin levels in sporadic and familial cases of canine narcolepsy. Neurobiol Dis 2001; 8(3):525–534.
49. Wagner JL, Storb R, Storer B, Mignot E.DLA-DQB1 alleles and bone marrow transplantation experiments in narcoleptic dogs. Tissue Antigens 2000; 56(3):223–231.
50. Fujiki N, Yoshida Y, Ripley B, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin-ligand-deficient narcoleptic dog. Sleep 2003; 26(8):953–959.
51. Schatzberg SJ, Cutter-Schatzberg K, Nydam D, Barrett J, Penn R, Flanders J, deLahunta A, Lin L, Mignot E. The effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. J Vet Intern Med 2004; 18(4):586–588.
52. Wu MF, Gulyani SA, Yau E, Mignot E, Phan B, Siegel JM. Locus coeruleus neurons: cessation of activity during cataplexy. Neurosci 1999; 91(4):1389–1399.
53. Wu MF, John J, Boehmer LN, Yau D, Nguyen GB, Siegel JM. Activity of dorsal raphe cells across the sleep-waking cycle and during cataplexy in narcoleptic dogs. J Physiol 2004; 554(Pt 1):202–215.
54. John J, Wu MF, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 2004; 42(4):619–634.
55. Tonokura M, Fujita K, Morozumi M, Yoshida Y, Kanbayashi T, Nishino S. Narcolepsy in a hypocretin/orexin-deficient chihuahua. Vet Rec 2003; 152(25):776–779.

24
Vigilance Tests in Narcolepsy

JOHANNES MATHIS and CHRISTIAN W. HESS
Department of Neurology, University Hospital, Bern, Switzerland

Sleepiness is a basic physiological need comparable to hunger or thirst, which is satisfied by sleeping, eating, or drinking and thus serves survival of the individual organism. Physiological sleepiness increases while being awake and underlies a circadian rhythm according to the two-process model. The state of sleepiness or drowsiness is a condition between wakefulness and overt sleep. The behavioural indicators are yawning, reduced activity, ptosis, eye rubbing, head drooping and the like. Sleepiness is a complex condition with different causes and consequences, comparable to pain. Whether sleepiness is uni-dimensional, varying only in severity, or multidimensional varying displace separation sign and change word sequence also qualitatively depending for example on REM- or NREM sleep pressure or related on tasks dependent alerting factors, has not yet been clarified (1).

Prevalence of excessive sleepiness rates up to 15% were reported in young adults and elderly people. Besides narcolepsy, EDS is also a predominant symptom in the sleep insufficiency syndrome, irregular sleep-wake rhythm (shift work, jet lag), sleep apnea syndrome (SAS), idiopathic hypersomnia and atypical depression (hypersomnolent depression). It is noteworthy that patients with insomnia suffer more from fatigue than from sleepiness during the day, in that they are not able to fall asleep when given the opportunity to do so. A rough grading of sleepiness into "mild," "moderate," and "severe" has been proposed in the International Classification of Sleep Disorders (2). It is generally assumed that EDS in narcolepsy is on average more severe than in other conditions of hypersomnia, but type and severity of EDS show great variability also among narcoleptic patients. Whether EDS in narcoleptics qualitatively differs from EDS due to other conditions is still a matter of debate. In clinical use the "narcoleptic type" of EDS, often described as irresistible, occurs also in active situations and is refreshing.

I. Pathophysiology and Etiology of EDS

Theoretically, the causes of sleepiness can be divided into two major categories, those which increase sleep pressure (REM and NREM) and those that reduce the wakefulness maintaining energies. Sleep pressure on one hand includes the homeostatic and circadian factors and sleep inertia. Factors modulating the capacity to maintain wake include individual motivation, task derived physical and intellectual activation,

monotony, temperature, light conditions, whole body vibrations, and heavy meals. These factors are not regarded as direct causes of EDS, but rather unmask an underlying increased sleep pressure. The subjective feeling of sleepiness can only be described by the individual and is not amenable to direct measurement. The measured behavioural indicators such as shortened sleep latency after lying down as measured by the multiple sleep latency test (MSLT), the struggle to remain awake, decreased performance levels, slowed cognitive function, and accidents are always the product of sleep pressure, reduced wakefulness maintaining energies, and environmental modulators.

Since sleepiness and wakefulness combine to a rather complex picture, how then can sleepiness or wakefulness be assessed? We should learn not to search for the one gold standard assessing method, but rather search for the optimal test battery with respect to the individual situation. In order to choose the appropriate methods, one first must always inquire after the goal of an assessment: Is it to establish (*i*) the presence of, or (*ii*) absence of sleepiness, or (*iii*) to monitor changes in sleepiness consecutively in a given patient? Furthermore, we must consider the actual purpose of the assessment: Is it for (*iv*) clinical purposes, (*v*) research, or (*vi*) for medico-legal purposes such as assessing fitness to drive? Finally and most importantly we must always consider the possibility of unspoken or ulterior motives: Are there psychological factors, or is there even a hidden agenda aiming at a (*vii*) primary or (*viii*) secondary gain of the disorder (e.g., malingering narcolepsy to get access to amphetamines or pretending good alertness to get the driver's licence back)?

II. Questionnaires

The history obtained by the experienced sleep specialist including the interview of a partner is certainly the most important source of information to reach a comprehensive judgement of EDS in the clinical context. Standardised scales are specifically designed to assess sleepiness and also help to distinguish sleepiness from fatigue.

The Epworth Sleepiness Score (3) (ESS) is at present the most widely used subjective sleepiness scale in clinical practice (Table 1). This questionnaire is based on the likelihood of falling asleep, which has to be rated by the patient for eight different social situations. In early publications (3,4) a good correlation (rho >0.5) of the ESS with the MSLT enabled the authors to conclude that the scale gives a valid estimate of sleep propensity in adults. In subsequent studies, however, the correlation between the ESS and the MSLT was shown to be weak (rho ~ 0.3) (5) or absent (6), and the same was true for the maintenance of wakefulness test (MWT) (7). Yet, this lacking correlation should not be taken as a shortcoming of these tests, but rather as pointing at the different facets of sleepiness which are differentially assessed (7). The ESS reflects an average level of sleepiness in terms of a temporal integral related to the preceding weeks, whereas the MSLT and MWT are more like snapshots relating to the short period during which they are performed. Therefore in a clinical setting one cannot rely on a single method of assessing sleepiness. We agree with Sangal et al. (7) that more than one method is required in making clinical decisions.

It is obvious that most questions of the ESS do not refer to the situation of an MSLT: The MSLT designed to directly measure "sleep propensity" is done with the

Table 1 The Epworth Sleepiness Scale (ESS)

Situation	Chance of dozing
How likely are you to doze off or fall asleep in the following situation, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation:	
0 = would never doze	1 = slight chance of dozing
2 = moderate chance of dozing	3 = high chance of dozing
1 Sitting and reading	—
2 Watching TV	—
3 Sitting in a public place (e.g. theater or a meeting)	—
4 As a passenger in a car for an hour without a break	—
5 Lying down in the afternoon when circumstances permit	—
6 Sitting and talking to someone	—
7 Sitting quietly after lunch without alcohol	—
8 In a car, while stopped for a few minutes in the traffic	—

Source: From Ref. 3.

subject passively lying in a bed, in a dark room, explicitly allowed to fall asleep. The popularity of the ESS is due to its simplicity and brevity and the fact that the test can be done by the patient without help of the medical doctor. Furthermore, it showed a good test-retest reliability, did correlate with other subjective sleepiness scales, and revealed an improvement in treatment studies of sleep apnea patients and patients with narcolepsy (8). The ESS correlates negatively with health related quality of life scale in SAS (9) and correlates positively with the likelihood of falling asleep at the wheel (10) and with the risk of suffering a work injury (11). This underlines the usefulness of this simple instrument in practical medicine, as long as it is used in the context of the clinical picture and along with complementary vigilance tests. One disadvantage is that the test is not useful for readministration in short intervals for example, when evaluating circadian sleepiness. No studies using the ESS have shown a clear group difference between sleepiness in narcolepsy and other causes of EDS, although the average score in narcolepsy is often among the highest of all patient groups (3).

The Stanford Sleepiness scale (SSS) (12) is based on a Likert self-rating scale with seven degrees of severity (Table 2). This method can be applied repetitively to

Table 2 The Stanford Sleepiness Scale

1 Feeling active and vital, alert, wide-awake.
2 Functioning at high level, but not at peak, able to concentrate.
3 Relaxed, awake, not full alertness, responsive.
4 A little foggy, not at peak, let down.
5 Fogginess, beginning to lose interest in remaining awake, slowed down
6 Sleepiness, prefer to be lying down, fighting sleep, woozy.
7 Almost in reverie, sleep onset soon, lost struggle to remain awake.

Source: From Ref. 12.

assess the momentary subjective (introspective) sleepiness and can even be repeated at short intervals, for instance to study circadian sleepiness. Comparisons between subjects' or patients' groups using the SSS are problematic, since normative data do not exist. The Karolinska Sleepiness Scale (13), and the visual analogue scale are other possibilities to assess subjective sleepiness. Cognitive test procedures are also sensitive to sleep deprivation and to fluctuations of arousal in narcolepsy (14), but these tests need specific knowledge and are not suitable for standardised bedside tests.

III. Multiple Sleep Latency Test (MSLT)

The MSLT consists of a series of four to six nap opportunities at two-hour intervals during the day, beginning approximately two hours after morning awakening. The test measures the propensity for falling asleep in a comfortable situation lying in bed in a dark and quiet room with the explicit permission to fall sleep. Two different versions of MSLT exist, a clinical and a research version (15). In the research version the accumulated sleep during the tests is minimised by always awakening the sleeper after sleep onset, defined as either occurrence of one epoch of sleep stage 2 to 4 or REM sleep or occurrence of three subsequent epochs of sleep stage-1. In the clinical version, the patient is not awakened after sleep onset, because a second objective of the test is to detect possible early REM sleep, so called sleep onset REM periods (SOREM). If a REM sleep episode occurs within 15 minutes after sleep onset, it is defined as SOREM. Therefore, each test session continues for 15 minutes after sleep onset, defined here as one epoch of any sleep stage. If no sleep occurs, the nap opportunity is terminated after 20 minutes in both versions of the MSLT.

The MSLT has sometimes been considered to be the "gold standard" for measuring sleep pressure (16). However the standard polysomnography, which has to be performed prior to the MSLT, does usually not take into account timing and duration of the individual sleep duration, which in turn can affect the MSLT result, particularly in long sleepers. For this reason it is useful to have the patient keep a sleep diary (16), and this should be done one week prior to the MSLT, since MSLT values can be influenced by sleep loss up to seven nights beforehand (17). A simultaneously performed actigraphy additionally helps to detect unusual sleep-wake habits.

Several case series of MSLT in normal controls of various ages and patients with EDS due to various causes have been published. An influence of age on sleep latency was found by some and not found by others. In narcolepsy an increase of the mean sleep latency with age was demonstrated (18). An average sleep latency of 5 minutes or less is assumed to indicate abnormal sleepiness, while an average sleep latency of over 10 minutes is considered normal with a diagnostic grey area between 5 and 10 minutes. As expected, the sleep latency as assessed by the MSLT correlates with the sleep latency of polysomnography. Otherwise, correlation between MSLT and test values of sleep quality obtained by polysomnography or subjective scores of EDS in sleep apnea syndrome (SAS) and narcolepsy were found to be weak or absent. Situational arousal could explain some discrepancies between MSLT results and subjective sleepiness scores in other disorders (19). Therefore, the debate on what is actually measured by the MSLT, and whether it should be taken as the gold standard for sleepiness, still continues (20).

The MSLT has only limited value for diagnosing a specific EDS causing disorder. Nevertheless, clearly abnormal sleep latencies of less than five minutes are most often found in narcolepsy (21), whereas the sleep latency of sleep apnea syndrome, idiopathic hypersomnia (21), or sleep insufficiency syndrome (22) more often fall in the "grey area" range between 5 and 10 minutes. On the other hand, the longest latencies are found in insomnia patients (23) and in patients with EDS due to depression (24).

A hallmark of narcoleptic sleep is the occurrence of sleep onset REM periods (SOREMP), that is, REM sleep within 15 minutes after sleep onset as first described by Vogel et al. (25). If in a narcoleptic patient no unambiguous cataplexy can be distinguished, the finding of two or more SOREMPs in the MSLT can be a critical diagnostic feature in favour of narcolepsy. Although an MSLT with ≥ 2 SOREMPs and < 5 min mean sleep latency indicates narcolepsy with a sensitivity of 70% and specificity of 97%, 30% of subjects with this combination have been reported not to have narcolepsy (26). These features were also found in 4.7% to 25% of sleep apnea patients (27). Only recently has it been shown that in narcolepsy the number of SOREMPs declines with increasing age (18), which might explain some of the discrepancies. In summary, it can be concluded that the MSLT results typical of narcolepsy are neither sufficient nor obligatory to diagnose narcolepsy, and it should be stressed that the MSLT must be interpreted in conjunction with the clinical and other paraclinical findings.

In narcolepsy some MSLT studies could document a weak (28) and others no significant treatment effect despite clinically effective dosage of the analeptic drugs.

IV. Limitations of the MSLT

There are essentially two critically discussed aspects of the MSLT: (*i*) While the MSLT seems suitable to assess sleep propensity as such, it is not the appropriate method to assess the ability to stay awake if required—that is, to judge the suitability for driving or fitness for duty. In order to answer this question, most experts would rather rely on the maintenance of wakefulness test (see below). Likewise, the inability of the MSLT to detect a possible therapy induced improvement of sleepiness in narcolepsy is a significant shortcoming.

(*ii*) A methodologically critical point is the definition of sleep onset in the MSLT. According to the official guidelines (16,29) sleep latency should be measured from lights off to the appearance of the first sleep epoch that is, 30 seconds of sleep stage-1. However, to be on the safe side, several experts require 30 seconds of "unequivocal sleep," that is sleep stage 2, 3, 4, or REM or alternatively three consecutive epochs of sleep stage-1. On the other hand, depending on the objective of the test, the one sleep stage-1 epoch criterion could perhaps also be too strict to be sensitive enough (30). It is, for instance, conceivable that the weak or absent correlation between subjective sleepiness scores and MSLT is due to the fact that the so called "micro-sleeps," with a duration of less than 15 seconds of sleep stage-1, are not taken into account by the routine MSLT assessment. The R&K criteria ignore states of drowsiness or sleepiness when moving from wakefulness to R&K NREM stage-1, which is particularly dissatisfying in the MWT. In order to close this gap, an adapted scoring method has been proposed (31) using a minimal "epoch duration" of 0.5 second and including several stages of drowsiness.

(*iii*) It is evident that by deliberately or perhaps subconsciously resisting to fall asleep, the sleep latency of an MSLT is falsely prolonged with the possibility of a false negative result.

V. Maintenance of Wakefulness Test (MWT)

This test is now frequently used to assess the ability to stay awake in cases where the suitability for driving (32) or fitness for duty is questioned (33). The subject is usually sitting rather than lying in a bed and, most importantly, is instructed to stay awake. The original test was performed in trials of 20 minutes, but later some experts proposed 40 minutes instead because ceiling effects were observed with the 20 minutes trials. Some experts used a latency criterion of one epoch of any stage (34), whereas in later studies the criterion of three stage-1 epochs was used (32,35). With either version, the MWT has now been applied to numerous patients with narcolepsy (35), SAS (32), or both (34). The first systematic study to get normal values was performed by Doghramji et al. in 1997 (36). Similar values have been obtained in an Australian study in randomly recruited 31 healthy subjects (37), although they used much brighter light conditions (1 lux). In a large multi-centre treatment trial on patients with narcolepsy free of psychoactive drugs (28), the 20 minutes version of MWT with four trials during the day revealed a mean sleep latency of $6.0 +/- 4.8$ minutes to sustained sleep. Only 1.5% of all narcoleptics were able to remain awake during all four 20-minutes trials compared to 55% of normal controls in Doghramji's study, and 14.5% of the narcoleptics had a mean latency of >12 minutes as compared to 95% of the normal controls. The sleep latency in narcoleptics was found to be inversely related to the severity of cataplexy, hypnagogic hallucinations, and sleep paralysis but age, gender, and duration of illness did not influence sleep latency.

Preliminary propositions for requirements of driving ability of >15 minutes mean sleep latency assessed by MWT have been proposed (32,33). However, in contrast to this rather low limit, we agree with other experts, who demand—at least for professional drivers - (taxi, bus, lorry, pilots, engine) a much higher limit of >30 or even 40 minutes as prerequisite for allowing a patient to drive (M. Partinen, J. Horne, personal communications). Since no pertinent studies are available correlating the MWT results with the risk of motor vehicle crashes, a well-founded limit of MWT measured mean sleep latency cannot be proposed yet.

A second indication of the MWT is assessment of treatment effects, for which the MWT has been shown to be more suitable than the MSLT (29,33,38). Most of the more recent treatment trials in narcolepsy have used the MWT to objectively measure the treatment effect (28).

Direct comparison between the MWT and the MSLT performed at the same day (33,34) showed only a weak correlation between MSLT and MWT results ($rho = 0.41$). Variance of the MWT values accounted for only 16% of the variance of MSLT values, indicating that the test results were relatively independent. Low to inexistent correlations between different vigilance tests were also found in our own analysis on 230 patients with EDS due to various conditions (Fig. 1). From these data it has become apparent that sleepiness and alertness cannot be considered as mere reciprocal qualities (33). It must, on the contrary, be concluded that subjective sleepiness and lack of

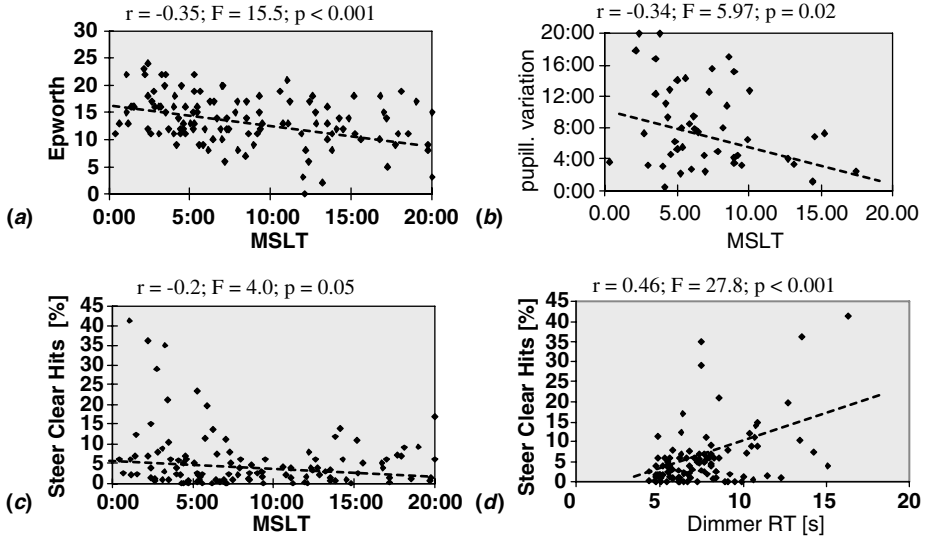


Figure 1 Comparison between vigilance tests in a patient samples of 230 patients referred to the sleep disorders center in Bern because of EDS were investigated using Epworth scale, MSLT, steer clear, Dimmer test (a self devised reaction time test with gradual appearance of the visual stimulus) pupillography subsequent to polysomnography the preceding night (not all patients had all tests). Note the rather low or absent correlations between tests.

alertness both include several components, based on various brain mechanisms: (*i*) The ability to fall asleep when allowed to do as assessed by the MSLT, (*ii*) the inability to stay awake when required to as measured by the MWT, (*iii*) a reduced attention as measured by cognitive neuropsychological performance tests, reaction time tests, driving simulators, and long latency evoked potentials, (*iv*) fatigue in the sense of tiredness or loss of energy ascertainable only by subjective tests, (*v*) fatiguability in the sense of a time-on-task performance decrement which may be a separate component or a complex composite of all other components. The MWT is of course not immune to the theoretical risk of falsification, when using it for diagnosis of EDS: If a subject deliberately does not resist falling asleep, a false positive result can result.

To obtain a more complete picture a combination of the MSLT with the MWT on the same day was suggested, but reducing the number of MSLT trials too much also reduced its reliability. In addition, the clinical version of the MSLT allowing up to 15 minutes of sleep may degrade the result of the subsequent MWT. We propose alternating MSLT and MWT procedures on the same day only for diagnostic purposes, but not when medico-legal issues about alertness and fitness are at stake.

VI. Reaction Time Tests

In a simple reaction time test ("Steer Clear") on PC basis a two lane street is presented, and the subject has to press a button to avoid hitting obstacles which appear randomly

on either lane during a 30 minutes test duration. The test is equivalent to a "go" and "no go" reaction time paradigm, but instead of measuring reaction time the number of performance failures ("hits") is counted in percentage of all obstacles. This represents the frequency of "lapses" corresponding to reaction times above a certain level. They showed an abnormally poor test performance in 16 narcoleptics (39) and demonstrated that this poor result was associated with a higher rate of car accidents as reported by the department of motor vehicles during a 5 years period. Under treatment with stimulants, the error rate in narcoleptics was reduced to normal levels.

The Oxford Sleep Resistance test (OSLER), developed as a substitute for the MWT, uses a behavioural element to determine sleep onset (40). The subject has to press a switch in response to the flash of a light emitting diode (LED), lightening up every three seconds. Sleep onset is defined as the failure to respond to the light at seven consecutive LED illuminations. The test could discriminate SAS patients from normal subjects.

The psycho-vigilance test (PVT) is another simple visual reaction time test (41) with continuous feed back information on reaction time. The number of lapses, defined as reaction times greater than 500 msec, are counted as measure of reduced performance. The test is sensitive to circadian changes of sleepiness and effects of sleep deprivation in healthy subjects (42), to night shift effects, and to effects of CPAP treatment in SAS despite its short duration of only 10 minutes. False positive results may be seen in cases of low motivation and attention deficit due to neuropsychological disorders.

VII. Pupillography

Several studies showed that the diameter of the pupil is inversely and its variability over time is positively related to subjective complaints of sleepiness (43). The method has been used mainly in a clinical environment to assess EDS because it requires little cooperation hence being very objective. It was shown to be sensitive to sleep restriction in healthy subjects (43). The method gives reliable results when comparing sequential tests in the same individual, but seems less suitable to compare one subject with another (15). Normative data are sparse, and consequently the technique has not come into general use for evaluation of EDS. It was repetitively applied in narcolepsy showing that instability that is, the variance of the pupil diameter might be a more sensitive measure to detect EDS than the mean pupil diameter as such (44).

VIII. Driving Simulators

Patients with EDS are at higher risk of motor vehicle accidents due to falling asleep at the wheel (45), and a large proportion of motor vehicle accidents of a driving population are due to sleepiness (46). Various sophisticated driving simulators exist with the aim to answer the crucial question of whether a patient with EDS (or other impairments) is fit to properly drive a motor vehicle or not. Particularly when testing professional drivers such "realistic" test procedures are indicated, but it goes beyond the scope of this chapter to describe them.

IX. Continuous Ambulatory EEG Monitoring

In narcoleptic patients ambulatory 24 hour EEG recording was first performed by Broughton et al. (47) showing a greater amount of sleep and drowsiness episodes during daytime compared to normal controls, associated with lapses of performance. Interestingly, the amount of daytime sleep did not correlate with MSLT measures.

Continuous ambulatory EEG recording proved to be more informative than the MSLT detecting a greater number of SOREM's in narcoleptics (48), but no control group was included in this study. Continuous recording over 24 hours or more, combined with MSLT testing, may also give useful information on the total sleep duration needed by an individual patient, and this can help to differentiate between long sleepers, idiopathic hypersomnia, and other poorly defined diagnostic entities.

The transition phase between wakefulness and sleep, the presleep period, was also specifically investigated by using EEG technique in healthy subjects and patients (49). It could nicely be shown that respiratory, subjective, and performance changes were most important between stage "wakefulness" and stage NREM 1, supporting the concept of a sleep onset period rather than of a sleep onset time point in this "no man's land" between wakefulness and overt sleep. Unfortunately the most widely used sleep scoring criteria proposed by Rechtschaffen and Kales do not allow an analysis of the microstructure of this period with sufficient time and space resolution, and do also not include "drowsiness stages." EEG recording with a full 10-20 electrode array system allows topographical assessment of EEG changes during the states of drowsiness. The sub-harmonic alpha rhythm, the "anterior alpha of drowsiness," the midline 4 to 5 Hz theta rhythms, the posterior occipital transients of sleep (POSTS), rhythmic mid temporal discharges (RMTD) are all elements associated with drowsiness, escaping the sole central recordings at C3/C4. Some sleep typical elements such as vertex waves, K-complexes, saw tooth waves, and even sleep spindles are better detected at Cz than at the parasagittal central electrodes C3 and C4, and therefore the recommendations made by Rechtschaffen and Kales are not suitable to describe states of drowsiness adequately.

X. Complex Event Related Potentials

In a group of hypersomniac patients no or only weak (rho <0.12) correlations were found between visual or auditory P300 amplitude and sleep latency on the MSLT or MWT (50). Variable results have also been described in narcolepsy, and the method was less reliable in discriminating narcoleptics from normal controls than the MSLT (51).

XI. Actigraphy

Actigraphy cannot be used to assess sleepiness at a specific time of the day. However, the inactivity periods, which can be objectively recorded over several days, can help to define an increased "time in bed" which could be a consequence of "hypersomnia." Distinction from liability to remain in bed due to depression or chronic fatigue syndrome must however be based on additional clinical information.

Table 3 Cardinal Test Methods of EDS in Narcolepsy

Test	Main indications	Strengths	Limitations/drawbacks
MSLT	Diagnosis of EDS (sleep propensity) in a sleep promoting environment	Long-standing experience in several disorders Reliable normal values Detection of SOREMs	Various definitions of sleep onset, false negative results possible (e.g., deliberately restisting sleep), no testing for fitness for duty, several test versions in use, weak correlation with subjective sleepiness
MWT	Resistive capacity not to fall asleep despite EDS, fitness for duty/driving monitoring treatment	Assessing fitness for duty, normal values are available since recently	False positive results possible (e.g., deliberately not resisting falling asleep), weak correlations with performance tests
Epworth SS	Subjective (average) sleepiness	Quick and easy to perform, correlates with other subjective scores Separates EDS from fatigue (loss of energy)	Not applicable in short sequences, variable correlations with objective tests (questions refer to both, sleep promoting situations and sleep resisting i.e., active situations)
Stanford SS Karolinska SS Visual Analogue S	Subjective (momentary) sleepiness	Quick and easy to perform, Applicable in short sequences	No reliable normal values, differentiation between EDS and fatigue or depressed state uncertain
Reaction time tests/ performance tests	Ability to perform correctly in a (divided) attention task despite EDS, fitness for duty/driving	Direct assessment of attention Performance in a specific task	Complex driving simulators require technical skills, impossible differentiation from other causes of impaired attention, false positive results possible depending on cooperation
Pupillography	EDS in almost inactive quiet wakefulness	Rapidly performed when equipment installed, easy to understand by the patient	Situation rarely comparable to daily life conditions, affected by eye lid drop and by autonomic system diseases

XII. Assessment of Sleepiness in Children

For the assessment of sleepiness in adolescent children the same tools as in adults can be used, but in younger children age dependent normal values must be used. The mean sleep latency in the MSLT ranges from $18.8 +/- 1.8$ in Tanner stage I to $15.8 +/- 3.5$ in older adolescents (52). In the largest series of MSLTs in narcoleptic children Guilleminault and Pelayo (53) found at least two or more SOREMs, but in smaller series false negative and false positive finding were reported (overview in (54)). For children, a pictorial sleepiness scale containing five faces representing various degrees of sleepiness and vigilance, ranking from greatest wakefulness to sleep, was designed (55), which can be used instead of the ESS.

XIII. Summary and Perspectives

Sleepiness can be assessed by subjective and objective methods, but correlations between the corresponding tests within the same subject and even more between subjects are weak or in-existent. This might partly have methodological reasons and for example, be due to inappropriate definitions of the measured parameters. For example, the definition of sleep latency until the occurrence of 15 to 45 seconds of sleep stage 1 might be adequate to characterise sleep latency in nocturnal polysomnography. However, in a maintenance of wakefulness test, where fitness for working or fitness for driving is judged, shorter "micro-sleeps" and states of drowsiness should be considered as well. Another reason for the poor correlations between vigilance tests could lie in the different aspects of sleepiness which are assessed by the various tests. This could also explain the slightly better correlations between similar "active" tests (Fig. 1*d*) than between "active" performance tests and "passive" MSLT. Sleep propensity should perhaps be differentiated into NREM and REM sleep pressure. Similarly, the maintenance of wakefulness energies are influenced by multiple factors such as motivation, demands of the task, and the surrounding conditions (1).

It is, therefore, essential to assess alertness and sleepiness by a battery of tests (Table 3) tailored to the specific clinical problem, using questionnaires, sleep diaries, objective passive (MSLT) or active (MWT) vigilance and performance tests, as well as actigraphy. The results must always be validated in the clinical context, considering the likely cause of EDS, the personality and severity of EDS as well as the potential consequences or risks of EDS (56). It is not yet clear whether continuous EEG recording and automatic EEG analysis (power spectra) will provide more sensitive and more specific information than careful visual assessment of a given vigilance test where EEG, EOG, performances, and video supervision of sleepy behaviour can be combined.

In order to reduce costs and time it will never be possible to imitate real life perfectly when assessing EDS, and laboratory tests will always be necessary. Studies to correlate fitness for working or fitness for driving in real life with laboratory vigilance tests are urgently needed.

References

1. Broughton RJ, Valley V, Aguirre M, Roberts M, Suwalsky J, Dunham E. Excessive daytime sleepiness and the pathophysiology of narcolepsy-cataplexy. A laboratory perspective. Sleep 1986; 9:205–215.

2. American Academy of Sleep Medicine. International classification of sleep disorders, revised: Diagnostic and coding manual. Rochester, Minnesota: Johnson Printing, 2000.
3. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14:540–545.
4. Johns MW. Sleepiness in different situations measured by the Epworth Sleepiness Scale. Sleep 1994; 17(8):703–710.
5. Olson LG, Cole MF, Ambrogetti A. Correlations among Epworth Sleepiness Scale scores, multiple sleep latency tests and psychological symptoms. J Sleep Res 1998; 7(4):248–253.
6. Benbadis SR, Mascha E, Perry MC, Wolgamuth BR, Smolley LA, Dinner DS. Association between the Epworth sleepiness scale and the multiple sleep latency test in a clinical population. Ann Intern Med 1999; 130(4):289–292.
7. Sangal RB, Mitler MM, Sangal JM. Subjective sleepiness ratings (Epworth sleepiness scale) do not reflect the same parameter of sleepiness as objective sleepiness (maintenance of wakefulness test) in patients with narcolepsy. Clin Neurophysiol 1999; 110:2131–2135.
8. American Sleep Disorders Association. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy: US Modafinil in Narcolepsy Multicenter Study Group. Neurology 2000; 54(5):1166–1175.
9. Chen NH, Johns MW, Li HY, Chu CC, Liang SC, Shu YH, et al. Validation of a Chinese version of the Epworth sleepiness scale. Qual Life Res 2002; 11(8):817–821.
10. Maycock G. Sleepiness and driving: The experience of U.K. drivers. Accid Anal and Prev 1997; 29:453–462.
11. Melamed S, Oksenberg A. Excessive daytime sleepiness and risk of occupational injuries in non-shift daytime workers. Sleep 2002; 25(3):315–322.
12. Hoddes E, Dement W, Zarcone V. The developement and use of the Stanford sleepiness scale (SSS). Psychophysiology 1972; 9:150–151.
13. Akerstedt T, Gillberg M. Subjective and objective sleepiness in the active individual. Intern J Neurosci 1990; 52:29–37.
14. Hood B, Bruck D. Sleepiness and performance in narcolepsy. J Sleep Res 1996; 5(2):128–134.
15. Mitler MM, Carskadon M, Hirshkowitz M. Evaluating Sleepiness. In: Kryger MH, Roth Th, Dement WC, eds. Sleep Medicine. Philadelphia: W.B. Saunders Company, 2004: 1251–1257.
16. Carskadon MA. Guidelines for the multiple sleep latency test (MSLT): A standard measure of sleepiness. In: Kryger H, Roth T, Dement WC, editors. Principles and pratice of sleep medicine. London: W.B. Saunders, 1997: 962–965.
17. Carskadon MA, Dement WC. Cumulative effects of sleep restriction on daytime sleepiness. Psychophysiology 1981; 18:107–113.
18. Dauvilliers Y, Gosselin A, Paquet J, Touchon J, Billiard M, Montplaisir J. Effect of age on MSLT results in patients with narcolepsy-cataplexy. Neurology 2004; 62(1):46–50.
19. Kronholm E, Hyyppa MT, Alanen E, Halonen JP, Partinen M. What does the multiple sleep latency test measure in a community sample? Sleep 1995; 18(10):827–835.
20. Chervin RD. The multiple sleep latency test and Epworth sleepiness scale in the assessment of daytime sleepiness. J Sleep Res 2000; 9(4):399–401.
21. Bassetti C, Aldrich MS. Idiopathic hypersomnia. A series of 42 patients. Brain 1997; 120:1423–1335.
22. Roehrs T, Zorick F, Sicklesteel J, Wittig R, Roth T. Excessive daytime sleepiness associated with insufficient sleep. Sleep 1983; 6:319–325.
23. Seidel WF, Dement WC. Sleepiness in insomnia: evaluation and treatment. Sleep 1982; 5 Suppl 2:S182–S190.
24. Reynolds CF, III, Coble PA, Kupfer DJ, Holzer BC. Application of the multiple sleep latency test in disorders of excessive sleepiness. Electroencephalogr Clin Neurophysiol 1982; 53(4):443–452.
25. Vogel G. Studies in the psychophysiology of dreams. III. The dream of narcolepsy. Arch Gen Psychiatry 1960; 3:421–425.
26. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 1997; 20(8):620–629.
27. Chervin RD, Aldrich MS. Sleep onset REM periods during multiple sleep latency tests in patients evaluated for sleep apnea. Am J Respir Crit Care Med 2000; 161(2 Pt 1):426–431.
28. Multicenter study group, Mitler MM, Guilleminault C, Harsh JR, Hirshkowitz M. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol 1998; 43:88–97.

29. Thorpy MJ. The clinical use of the multiple sleep latency test. Sleep 1992; 15:268–276.
30. Harrison Y, Horne JA. Occurrence of "microsleeps' during daytime sleep onset in normal subjects. Electroencephalogr Clin Neurophysiol 1996; 98(5):411–416.
31. Himanen SL, Saastamoinen A, Hasan J. Increasing the temporal resolution and stage specificity by visual adaptive scoring (VAS) - a preliminary description. Sleep and Hypnosis 1999; 1:22–28.
32. Poceta JS, Timms RM, Jeong D, Ho S, Erman MK, Mitler MM. Maintenance of wakefulness test in obstructive sleep apnea syndrome. Chest 1992; 101:893–897.
33. Sangal RB, Thomas L, Mitler MM. Disorders of excessive sleepiness. Treatment improves ability to stay awake but does not reduce sleepiness. Chest 1992; 102(3):699–703.
34. Sangal RB, Thomas L, Mitler MM. Maintenance of wakefulness test and multiple sleep latency test. Measurement of different abilities in patients with sleep disorders. Chest 1992; 101:898–902.
35. Mitler MM, Walsleben J, Sangal RB, Hirshkowitz M. Sleep latency on the maintenance of wakefulness test (MWT) for 530 patients with narcolepsy while free of psychoactive drugs. Electroencephalogr Clin Neurophysiol 1998; 107(1):33–38.
36. Doghramji K, Mitler MM, Sangal RB, Shapiro C, Taylor S, Walsleben J, et al. A normative study of the maintenance of wakefulness test (MWT). Electroencephalogr Clin Neurophysiol 1997; 103(5): 554–562.
37. Banks S, Barnes M, Tarquinio N, Pierce RJ, Lack LC, McEvoy RD. The maintenance of wakefulness test in normal healthy subjects. Sleep 2004; 27(4):799–802.
38. Mitler MM, Gujavarty KS, Browman CP. Maintenance of wakefulness test: A polysomnographic technique for evaluating treatment efficacy in patients with excessive somnolence. Electroencephalogr Clin Neurophysiol 1982; 53:658–661.
39. Findley L, Unverzagt M, Guchu R, Fabrizio M, Buckner J, Suratt P. Vigilance and automobile accidents in patients with sleep apnea or narcolepsy. Chest 1995; 108:619–624.
40. Bennett LS, Stradling JR, Davies RJ. A behavioural test to assess daytime sleepiness in obstructive sleep apnoea. J Sleep Res 1997; 6(2):142–145.
41. Dinges DF, Powell JW. Microcomputer analysis of performance on a portable, simple visual task during sustained operations. Behav Res Methods Instrum Comput 1985; 17:652–655.
42. Van Dongen HP, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 2003; 26(2):117–126.
43. Wilhelm B, Giedke H, Ludtke H, Bittner E, Hofmann A, Wilhelm H. Daytime variations in central nervous system activation measured by a pupillographic sleepiness test. J Sleep Res 2001; 10(1):1–7.
44. Newman J, Broughton RJ. Pupillometric assessment of excessive daytime sleepiness in narcolepsy-cataplexy. Sleep 1991; 14:121–129.
45. Horstmann S, Hess CW, Bassetti C, Gugger M, Mathis J. Sleepiness-related accidents in sleep apnea patients. Sleep 2000; 23(3):383–389.
46. Horne JA, Reyner LA. Sleep related vehicle accidents. Br Med J 1995; 310:565–567.
47. Broughton RJ, Dunham W, Newman J, Lutley K, Duschesne P, Rivers M. Ambulatory 24 hour sleep-wake monitoring in narcolepsy-cataplexy compared to matched controls. Electroencephalogr Clin Neurophysiol Suppl 1988; 70:473–481.
48. Genton P, Benlakhel K, Disdier P, Leprince Y, Lavernhe G, Viallet F, et al. [Diagnosis of narcolepsy-cataplexy: importance of continuous recording in ambulatory EEG. Report of 20 cases]. Neurophysiol Clin 1995; 25(4):187–195.
49. Alloway CE, Ogilvie RD, Shapiro CM. EEG spectral analysis of the sleep-onset period in narcoleptics and normal sleepers. Sleep 1999; 22(2):191–203.
50. Sangal RB, Sangal JM. Measurement of P300 and sleep characteristics in patients with hypersomnia: do P300 latencies, P300 amplitudes, and multiple sleep latency and maintenance of wakefulness tests measure different factors? Clin Electroencephalogr 1997; 28(3):179–184.
51. Broughton RJ, Aguirre M, Dunham W. A comparison of multiple and single sleep latency and cerebral evoked potential (P300) measures in the assessment of excessive daytime sleepiness in narcolepsy-cataplexy. Sleep 1988; 11(6):537–545.
52. Carskadon MA. The second decade. In: Guilleminault C, ed. Sleeping and Waking Disorders: Indications and Techniques. Menlo Park, CA: Addison Wesley, 1982: 99–125.
53. Guilleminault C, Pelayo R. Narcolepsy in prepubertal children. Ann Neurol 1998; 43(1):135–142.

54. Hoban TF, Chervin RD. Assessment of sleepiness in children. Semin Pediatr Neurol 2001; 8(4): 216–228.
55. Maldonado CC, Bentley AJ, Mitchell D. A pictorial sleepiness scale based on cartoon faces. Sleep 2004; 27(3):541–548.
56. Mathis J, Seeger R, Ewert U. Excessive daytime sleepiness, crashes and driving capability. Schweizer Archiv für Neurologie und Psychiatrie 2003; 154:329–338.

25
Lessons from Sleepy Mice: Narcolepsy-Cataplexy and the Orexin Neuropeptide System

JON T. WILLIE
Department of Neurosurgery, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

MASASHI YANAGISAWA
Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A. and ERATO Yanagisawa Orphan Receptor Project, Japan Science and Technology Agency, Tokyo, Japan

I. Introduction

Narcolepsy-cataplexy consists of two underlying problems: (*i*) inability to maintain wakefulness, and (*ii*) intrusion of features of REM sleep into wakefulness or at sleep onset. Together these result in the symptom complex of excessive daytime sleepiness accompanied by irresistible "sleep attacks," cataplexy, sleep-onset hallucinations, and sleep paralysis. A reliable diagnostic sign is that of "sleep-onset REM sleep" (SOREM) periods during polysomnographic recordings of daytime naps. Narcolepsy-cataplexy, in the vast majority of human cases, results from selective loss of hypothalamic neurons containing orexin (hypocretin) neuropeptides, possibly by an autoimmune process. The neuropeptides orexin-A and orexin-B are products of the *prepro-orexin* (*prepro-hypocretin*) gene that is expressed by a population of neurons in the lateral hypothalamic area (LHA). Notably, orexin neurons send projections throughout the brain and spinal cord with particularly dense innervations of monoaminergic and cholinergic centers controlling sleep-wake in the forebrain and brainstem. Orexin peptides are endogenous ligands for two G protein-coupled receptors termed orexin receptors type 1 and type 2 (OX1R and OX2R), and a number of studies have suggested that orexin peptides are primarily neuroexcitatory. OX1Rs exhibit moderately higher affinity for orexin-A while OX2Rs exhibit equal affinity for both orexin-A and orexin-B. The differential distribution of the two orexin receptors suggests distinct roles of each receptor in aspects of vigilance state control and muscle tone.

The structure and neurobiology of sleep are highly conserved among mammals, and the polysomnogram is amenable to study in laboratory rodents. Mice resemble humans genetically and physiologically, and they offer advantages over other animal models of disease: they can be genetically manipulated and are relatively cheap to maintain. This chapter reviews experimental results collected from two common types of genetically modified animals: knockout and transgenic mice. They include *prepro-orexin/hypocretin* gene knockout ($orexin^{-/-}$), *OX1R* gene knockout

($OX1R^{-/-}$), *OX2R* gene knockout ($OX2R^{-/-}$), double receptor gene knockout ($OX1R^{-/-}$;$OX2R^{-/-}$), orexin/ataxin-3 transgenic, and CAG/orexin;orexin/ataxin-3 double transgenic mice (Table 1).

II. Molecular Genetic Analysis of Narcolepsy-Cataplexy in Mice

Chemelli et al. (1). first noted that knockout of the *prepro-orexin* gene ($orexin^{-/-}$ mice) causes a phenotype remarkably similar to the human disorder: abrupt behavioral arrests with muscle atonia, fragmented wakefulness, and direct transitions from wakefulness to REM sleep (Table 1). Likewise, mice with a selective postnatal ablation of orexin-producing neurons (*orexin/ataxin-3* transgenic mice produced by genetic expression of a neurotoxic polyglutamine repeat driven by an *orexin* gene promoter element) have essentially identical behavioral abnormalities (2). Thus, while orexin neuron destruction results in narcolepsy-cataplexy in humans and mice, orexin peptide deficiency alone is sufficient to produce the symptom complex—a key pathophysiological observation.

However, the mechanisms by which absence of orexin signaling causes symptoms of narcolepsy-cataplexy are unknown. Despite the association of *OX2R* mutations with narcolepsy in dogs, the differential roles of OX1R and OX2R in the human symptom complex remain uncertain. While the Doberman model has been used to investigate the neurochemical substrates of cataplexy (3,4), it differs biochemically from the vast majority of human narcolepsy-cataplexy cases in which orexin peptides are lacking, and *OX2R*-mutant Dobermans suffer a milder form of the syndrome when compared to sporadic narcoleptic dogs that are likely to have been orexin-deficient (5,6). To address this problem in a controlled fashion, careful comparison of murine phenotypes was undertaken after detailed definition of behavioral abnormalities.

A. Characterization of Cataplexy

From human studies, the phenomenon of cataplexy has been conceptualized either as a fragmentary manifestation of REM sleep or, alternatively, as a transitional state between wakefulness and REM sleep (7). More recently, studies of brain regions involved in the triggering of cataplexy in *OX2R*-mutant Dobermans have emphasized the conception of cataplexy as a unique state not directly related to REM sleep, but probably sharing a final common pathway of muscle tone inhibition at the levels of brainstem and spinal cord (3,4).

Accepted clinical criteria for the definition of cataplexy in humans and a comparison to abnormalities observed in mice are shown in Table 2. Abrupt behavioral arrests in $orexin^{-/-}$, $OX2R^{-/-}$, and orexin neuron-ablated mice fulfill criteria used for human cataplexy (1,2,8). These arrests are discrete phases of postural atonia (Figure 1*a*) of short duration (seconds to minutes) that may be preceded by brief gait disturbances due to propagating atonia. Mouse cataplexy is triggered during active waking periods with emotional content, and it is specifically suppressed by clomipramine. Preservation of consciousness has also been documented following the onset of some arrests in mice (8).

Concurrent EEG/EMG/video recording and spectral analysis of cataplexy episodes in $orexin^{-/-}$ and $OX2R^{-/-}$ mice demonstrated that onsets of postural

Table 1 Genetically Modifed Mice Used in Behavioral and Sleep Studies of Narcolepsy-Cataplexy

Genetic modification	Pathophysiology	Relevant findings	Interpretations
Prepro-orexin (hypocretin) gene knockout (*orexin*$^{-/-}$)	Loss of orexin-A and -B function throughout development	Inability to maintain wakefulness *Severe* decrease in REM sleep latency *Frequent* cataplexy and direct transitions to REM sleep	Severe narcolepsy-cataplexy comparable to human form (1,8).
Orexin receptor type 1 gene knockout (*OX1R*$^{-/-}$)	Loss of OX1R function throughout development	*Mild* decrease in REM sleep latency Absence of cataplexy or direct transitions to REM sleep	OX1R signaling contributes to REM sleep gating (9,10).
Orexin receptor type 2 gene knockout (*OX2R*$^{-/-}$)	Loss of OX2R function throughout development	Inability to maintain wakefulness *Mild* decrease in REM sleep latency *Rare* cataplexy and direct transitions to REM sleep	Milder narcolepsy-cataplexy comparable to Doberman form. OX2R signaling stabilizes wake and contributes to REM sleep gating (8).
Double receptor gene knockout (*OX1R*$^{-/-}$;*OX2R*$^{-/-}$)	Loss of OX1R and OX2R function throughout development	Inability to maintain wakefulness *Severe* decrease in REM sleep latency *Frequent* cataplexy and direct transitions to REM sleep	Severe narcolepsy-cataplexy indistinguishable from *orexin*$^{-/-}$ mice (10).
Orexin/ataxin-3 transgenic mouse[a] (expression of neurotoxic gene fragment driven by orexin gene promoter)	Selective postnatal degeneration of orexin neurons complete by early adulthood	Inability to maintain wakefulness *Severe* decrease in REM sleep latency *Frequen*t cataplexy and direct transitions to REM sleep Phenotype reversed with intracerebroventricular administration of orexin-A	Narcolepsy-cataplexy comparable to human form and indistinguishable from *orexin*$^{-/-}$ mice (2). Pharmacological treatments based upon the orexin system likely to prove effective (35).

(*Continued*)

Table 1 Genetically Modifed Mice Used in Behavioral and Sleep Studies of Narcolepsy-Cataplexy *(Continued)*

Genetic modification	Pathophysiology	Relevant findings	Interpretations
CAG/orexin;Orexin/ataxin-3 double transgenic mouse (expression of *orexin* gene driven by hybrid promoter which drives widespread ectopic expression as well as *orexin/ataxin-3* transgene)	Widespread ectopic *prepro-orexin* expression in brain combined with selective degeneration of endogenous orexin neurons.	Improved ability to maintain wakefulness during active phase Absence of cataplexy or direct transitions to REM sleep	Ectopic production of orexin neuropeptides rescues narcolepsy-cataplexy (proof of concept for gene therapy) (35).

[a]The human *ataxin-3* gene fragment contains a neurotoxic polyglutamine repeat. Expression of the fragment by selective promoters results in targeted neurodegeneration in animals. The selective postnatal degeneration of neurons in *orexin/ataxin-3* transgenic mice (2) and rats (15) models the timing and specificity of the autoimmune degenerative process theorized to explain human narcolepsy-cataplexy. Nevertheless, orexin deficiency alone seems to be sufficient to reproduce the major symptoms of narcolepsy-cataplexy in mice.

Table 2 Comparison of Cataplexy

	Human	Mouse
Behavioral features	Sudden bilateral weakness of postural muscles[a]	"Abrupt" (onset <2 sec) discrete phases of postural atonia +/− preceding gait disturbance (1,8)
Level of consciousness	Preserved at onset (memory of episodes intact)[a]	Preserved at onset (detected by residual muscle response to visual stimuli) (1,8)
Provocation	Strong emotions (e.g., laughter)[a]	Active behavior with likely emotional content (e.g., running, climbing, vigorous grooming, social interaction) (1,8)
Duration	Brief (sec to min) unless transition to sleep occurs[a]	Brief (sec to min) (1,8)
EEG	Wake period alone or wake period → REM sleep if left undisturbed[b]	Direct transition to either REM sleep or REM sleep-like state in almost all cases (a period of wake EEG is usually imperceptible) (1,8)
EMG	Postural atonia at onset +/− myoclonic jerks in extremities	Postural atonia at onset +/− myoclonic activity in extremities ("rocking") (1,8)
Response to therapy	Suppressed by antidepressants with noradrenergic and anticholinergic activity, including clomipramine or imipramine[a]	Suppressed by clomipramine (8), and intracerebroventricular orexin-A (35)

[a]Criteria used to diagnose cataplexy clinically (40).
[b]EEG studies performed during cataplectic attacks have produced variable results in both humans and dogs (see preview review of this topic (8)), with some authors reporting wakefulness and others reporting REM sleep characteristics in the EEG, despite preservation of consciousness.

collapse were almost exclusively accompanied by direct transitions to REM sleep or the pre-REM spindling stage observed in mice (1,8). Transitions to REM sleep often occur during cataplexy in humans (7), but this phenomenon occurs rapidly and completely in nearly all cases of cataplexy in mice (8). Interestingly, that behavioral cataplexy is so closely associated with near instantaneous transition to REM sleep in this species tends to support the suggestion by Hishikawa and Shimizu that cataplexy is indeed a transitional state between wakefulness and REM sleep (7). However, brain sites and mechanisms for triggering cataplexy are distinct from those of REM sleep in the Doberman model (3), so the close association between cataplexy and REM sleep in mice may result from cataplexy, once entered, being a "back door" to REM sleep. That is, cataplexy and REM sleep may be independent phenomena, but cataplexy may be a state that leaves the animal highly vulnerable to expression of REM sleep.

When the frequency of cataplexy is compared across mouse lines, important differences are noted (Fig. 1*b*). As mentioned, *orexin*$^{-/-}$ mice and *orexin/ataxin-3* mice exhibit similar frequencies of cataplexy onset and spend similar amounts of time immobilized under defined experimental conditions during the first few hours

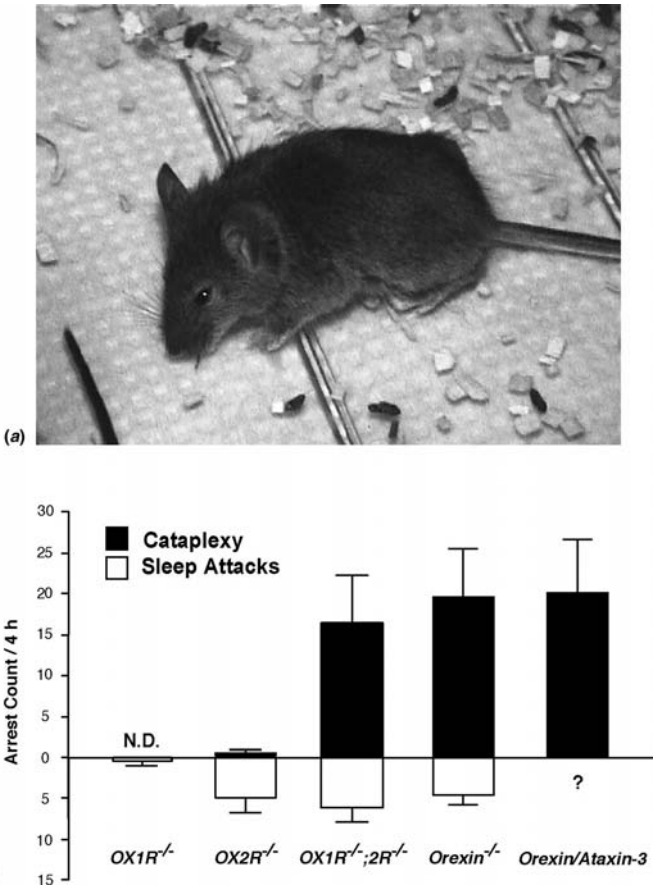


Figure 1 Cataplexy and sleep attacks in mice. (***a***) Image of an *orexin*$^{-/-}$ mouse during a cataplectic arrest. Cataplexy is reliably and specifically distinguished from normal behavior by using the following scoring criteria (1,2,8): (*i*) an abrupt cessation of purposeful motor activity over a period of <2 seconds (except where onset is prolonged by gait disturbances suggestive of partial cataplexy); (*ii*) a sustained postural collapse maintained throughout the episode; and (*iii*) an abrupt end to the episode with resumption of purposeful motor activity. By contrast, sleep attacks are distinguished from normal rest behavior and cataplexy using the following criteria (8): (*i*) a gradual cessation of purposeful motor activity over a period >2 seconds that is not preceded by stereotypical preparation for sleep (e.g., nesting and/or assumption of a curled or hunched posture with limbs drawn under the body) and may be accompanied by a characteristic bobbing of the head ("nodding-off"); (*ii*) a sustained postural collapse maintained throughout the episode; and (*iii*) an abrupt end to the episode with resumption of purposeful motor activity. (***b***) Frequencies of cataplexy and sleep attacks during part of the dark phase across mouse lines. Scoring was performed by blinded observers using behavioral criteria listed above. Mean frequencies and standard errors for 17 *OX1R*$^{-/-}$, 14 *OX2R*$^{-/-}$, 14 *OX1R*$^{-/-}$*;OX2R*$^{-/-}$, 12 *orexin*$^{-/-}$, and 6 *orexin/ataxin-3* transgenic mice are shown. No cataplectic or sleep attacks were identified in 26 wildtype littermate controls. Data are adapted from prior publications (2,8) as well as unpublished observations of *OX1R*$^{-/-}$ mice (Y. Y. Kisanuki and M. Yanagisawa). N.D., none detected. Question mark signifies unknown frequency of sleep attacks (this has not been reported in *orexin/ataxin-3* mice).

of darkness (the murine active phase). This deserves emphasis as these two models differ pathophysiologically (Table 1). In contrast, *OX1R*$^{-/-}$ mice *never* exhibit cataplexy (9,10), despite some confusion on this point in the literature (4). *OX2R*$^{-/-}$ mice, in contrast, exhibit cataplexy, but like Dobermans, they are only mildly affected relative to orexin-deficient animals (5,6,8). Indeed, in carefully controlled comparisons of *OX2R*$^{-/-}$ and *orexin*$^{-/-}$ mice, there is a roughly 30-fold difference in frequency of cataplexy (Figure 1*b*) and time spent immobilized (8). That *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ (double knockout) mice appear phenotypically equivalent to orexin-deficient mice, demonstrates that absence of signaling through both receptor pathways reproduces the severe cataplexy associated with orexin deficiency (10).

In addition to differences in cataplexy frequency, qualitative differences in cataplexy-associated phenomena among OX2R- and orexin-deficient mice have also been noted (8). For instance, *orexin*$^{-/-}$ and *orexin/ataxin-3* mice frequently exhibit rhythmic hindlimb myoclonic activity (stereotypical "rocking") during REM sleep that follows the onset of cataplexy (and sleep attacks, described below); *OX2R*$^{-/-}$ mice apparently do not (Table 2). The basis for and significance of this phenomenon is unknown; we speculate that rapid transitions to REM sleep in mice lead to insufficient inhibition of output from locomotor rhythm generators in the brainstem and/or spinal cord. This may explain the increased association between narcolepsy and REM sleep behavior disorder in humans (11). As *OX2R*$^{-/-}$ mice do not exhibit this rocking activity during attacks associated with REM sleep EEG, intact OX1R signaling may indirectly inhibit such generators or their output. While seemingly paradoxical when compared to the overall REM sleep-gating actions of orexin (discussed below), this may be consistent with the finding that orexin-A microinjections into different areas of pons modulate both facilitatory and inhibitory motor processes in decerebrate rats (12).

Conclusions regarding the underlying neuronal substrates for the onset and propagation of human cataplexy have largely been drawn from studies of OX2R-deficient Dobermans (3,4). However, differences in severity of cataplexy and cataplexy-associated phenomena between orexin- and OX2R-deficient narcolepsy models should provoke consideration of the possibility that the cascade of neurobiological events contributing to cataplexy may not be identical across pathophysiologically distinct animal models. Clearly, extracellular recordings in brain nuclei of freely moving mice will be needed to compare neuronal substrates of cataplexy in orexin-deficient and OX2R-deficient animals.

B. Characterization of Sleep Attacks

The sleep attacks of human narcolepsy are experienced as a phenomenon that is distinct from both cataplexy and normal sleep onset. Without access to the subjective experience of animals, attempts to probe behavioral abnormalities in narcoleptic animals can prove challenging. Nevertheless, mouse sleep attacks, scored using behavioral criteria different from cataplexy, have been described in detail for *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice (8). These events are comparable to human sleep attacks in several respects (Table 3). Such attacks frequently occur during quiet wake and are not associated with preceding gait disturbances or sudden muscle atonia suggestive of cataplexy. Unlike normal sleep, murine sleep attacks are not preceded by stereotypical

Table 3 Comparison of Sleep Attacks

	Human	Mouse
Subjective experience	Irresistible sleepiness not associated with abrupt muscle weakness (40). Prolonged attacks may be accompanied by sleep paralysis or hallucinations (7).	Unknown
Behavioral features	"Nodding off" during socially inappropriate circumstances	"Gradual" (onset $>$ 2 sec) loss of head and neck posture ("nodding off") (8)
Level of consciousness	Impaired consciousness and memory	Unknown
Provocation	Occur during quiet or inactive wake (meals, conversations, driving, etc.)	Associated with quiet wake (quiet grooming, slow ambulating, eating) (8)
Other association	Automatic behavior (chewing, driving) (41)	Automatic behavior (chewing) (8)
EEG	NREM sleep +/− SOREM period[a] (7)	*OX2R*$^{-/-}$: NREM sleep only (8) *Orexin*$^{-/-}$: NREM sleep +/− premature transition to REM sleep[b] (8)
EMG	Attenuated, but not atonic at onset	Attenuated, but not atonic at onset (8)
Response to therapy	Suppressed by psychostimulants	Supressed by caffeine (8), modafinil (21), intracerebroventricular orexin-A (35)

[a]SOREM period, sleep onset REM sleep period signifies $<$15 min of preceding NREM sleep in humans.
[b]In mouse, the term "premature transition to REM sleep" signifies $<$1 min of NREM sleep but is distinguished from "direct transition to REM sleep" observed in association with cataplexy in the mouse.

rest-associated behaviors such as nesting or normal murine sleep posture. They are associated with a loss of head and neck posture not unlike "nodding off" in sleepy humans and narcoleptic dogs (S. Nishino, personal communication, 2003). Importantly, wildtype mice do not exhibit these attacks under the same experimental conditions.

Frequencies of sleep attacks are alike among *orexin*$^{-/-}$, *OX2R*$^{-/-}$, and *OX1R*$^{-/-}$; OX2R$^{-/-}$ mice of similar background strain (Fig. 1*b*), potentially indicating a similar pattern of sleepiness across these models. Notably, such attacks are only very rarely detected in some *OX1R*$^{-/-}$ mice (Y.Y Kisanuki and S. Tokita, personal communication, 2005). While this may suggest very mild sleepiness relative to other mice, further studies are needed.

Like sleep attacks in humans, onsets of mouse sleep attacks in *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice are consistently accompanied by NREM sleep in the electroencephalogram. Furthermore, murine sleep attacks (but not cataplexy) are suppressed by caffeine and may be accompanied by semi-purposeful automatic behavior during NREM sleep (8). In *orexin*$^{-/-}$ mice, a large proportion of sleep attacks are accompanied by

premature transitions from NREM sleep (after <60 sec) to REM sleep. These transitions resemble human SOREM sleep episodes, the polysmnographic *sine qua non* of human narcolepsy diagnosis. Yet sleep attacks differ electroencephalographically in $OX2R^{-/-}$ mice in that they are briefer in duration and do not exhibit these premature transitions (8).

Thus, absence of OX2R-dependent signaling appears to be sufficient to cause the same level of sleepiness (as judged by frequency of sleep attack onset) as is observed with orexin-deficiency. Yet, a profound difference in the gating of REM sleep during NREM sleep attacks appears to exist between the two models of narcolepsy. This likely indicates a contribution of the OX1R pathway to the gating of REM sleep. Strain-controlled comparisons of *orexin/ataxin-3* mice to *orexin* mice are still needed to define what effect the pathophysiological distinction between these models might have upon sleep attacks or related behavior.

C. Fragmentation of Sleep and Wake States

One of the fundamental problems of narcolepsy-cataplexy is the inability to maintain wakefulness. Under baseline experimental conditions, $OX2R^{-/-}$, $orexin^{-/-}$, and *orexin/ataxin-3* transgenic mice have normal amounts of wake and NREM sleep over the course of light and dark phases and over the 24 cycle (1,2,8,13). Nevertheless, these three lines all exhibit a striking inability to maintain long bouts of wakefulness with decreased durations of wake and NREM sleep episodes and increased episode frequencies of these states, especially at night (the murine active phase). In contrast, the sleep-wake patterns of $OX1R^{-/-}$ mice appear not to be highly fragmented, but this phenotype has not been fully described (9,10).

Fragmented behavioral patterns, possibly an indicator of sleepiness, are roughly equivalent in $OX2R^{-/-}$ and $orexin^{-/-}$ mice (8). This inability to maintain wakefulness is not likely to result simply from the disruptive influence of cataplexy and associated transitions to REM sleep, as these events are much less frequent in $OX2R^{-/-}$ mice. However, the similar frequencies of sleep attacks in $OX2R^{-/-}$ and $orexin^{-/-}$ mice, described above, are consistent with a similar degree of sleepiness in these two models. Likewise, during the day (the murine rest phase), decreased duration and increased frequency of wake were also observed to varying degrees, although significant effects were not observed across all studies (1,8).

After specifically accounting for direct transitions to REM sleep in $orexin^{-/-}$ mice, Mochizuki et al. reexamined the effect of orexin deficiency upon sleep and wake (13). These authors found evidence of short sleep cycles during the light period, and short bouts of sleep were accompanied by many more transitions among all states (during light and dark phases) compared to wild type mice (Fig. 2). With the exception of frequent cataplexy-associated direct transitions from wake to REM sleep, $orexin^{-/-}$ mice did not exhibit a relative bias for other transitions among wake, NREM, or remaining REM sleep. Thus, these results indicate that one of the fundamental problems of narcolepsy-cataplexy, the inability to maintain wakefulness during the active phase, is also associated with inability to maintain normal sleep cycles during the rest phase. This is consistent with disturbed nocturnal sleep in narcoleptic patients (14).

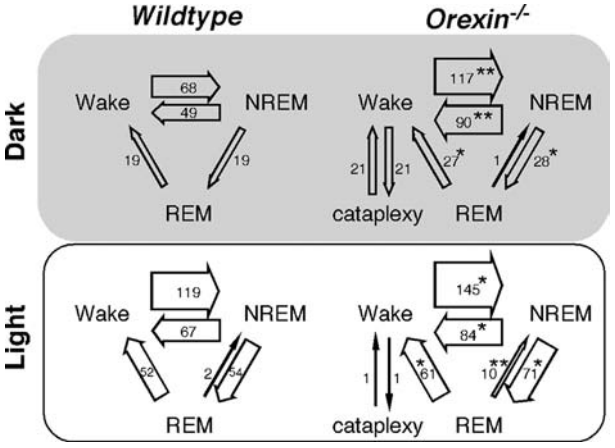


Figure 2 *Orexin*$^{-/-}$ mice have more transitions between all behavioral states. The mean number of transitions between states is indicated along the arrows between states and by the thickness of the arrows. All transitions are significantly increased in *orexin*$^{-/-}$ mice, and transitions into cataplexy account for only a small proportion of the transitions out of wakefulness. Note that cataplexy transitions are defined operationally in this analysis as direct transitions from wakefulness to REM sleep as this is a reliable indicator of the frequency of cataplexy in this species (8). $^{*}p < 0.05$; $^{**}p < 0.01$. *Source*: Adapted from Ref. 13.

D. Orexin as a Gatekeeper of REM Sleep

The second fundamental problem of narcolepsy-cataplexy is abnormal intrusion of REM sleep into wakefulness. Orexin-deficient mice, in 24-hour ambulatory recordings, exhibit frequent transitions from wake to REM sleep, especially during the dark phase. In contrast to the effects of fragmented behavioral states that cause reduced NREM sleep and wake durations in *orexin*$^{-/-}$ and *OX2R*$^{-/-}$ mice as described above, the frequent REM sleep episodes of *orexin*$^{-/-}$ mice are not abnormally brief. Indeed, cataplexy-associated REM sleep episodes and otherwise normal REM sleep episodes were often longer in duration than those of wildtypes (note the illustrative hypnograms in *orexin*$^{-/-}$ (1,8) and *orexin/ataxin-3* mice (2)). Also of note, orexin- and orexin-receptor deficient animals all exhibit significantly reduced latencies to REM sleep after the onset of sleep (1,2,8–10). These latencies are most severely reduced in *orexin*$^{-/-}$ and *orexin/ataxin-3* mice, lines exhibit frequent direct transitions to REM sleep during wakefulness. Nevertheless, *OX1R*$^{-/-}$ mice exhibit shortened latencies despite never having such transitions (Y.Y. Kisanuki and C.M. Sinton, personal communication, 2004).

Intrusions of REM sleep in orexin-deficient mice result in significantly higher levels of REM sleep over the dark (active) phase (1,8) (Fig. 3). Remarkably similar effects were recorded over the daytime in narcoleptic humans relative to normal individuals (14). Notably, REM sleep time in *OX1R*$^{-/-}$*;OX2R*$^{-/-}$ mice (10) was indistinguishable compared to *orexin*$^{-/-}$ mice. Isolated deficiencies in either OX1R or OX2R also resulted in tendencies toward increased active-phase REM sleep, but the effects were not statistically significant in these studies (8–10).

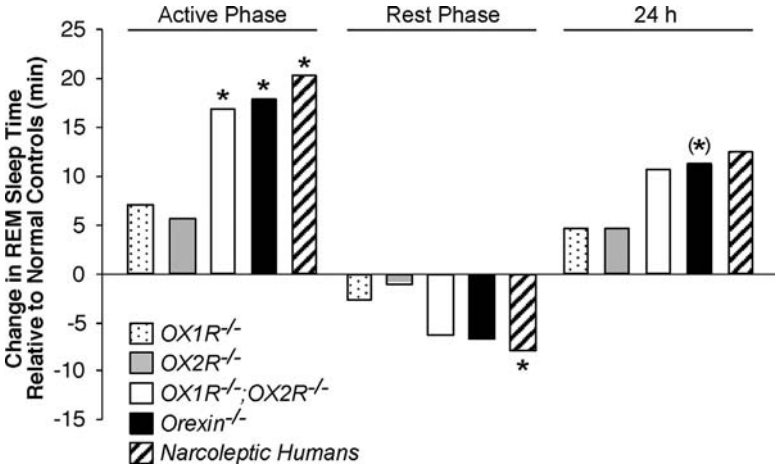


Figure 3 Changes in REM sleep amounts relative to control groups across phases (active, rest, or 24 hours) in knockout mice and narcoleptic humans. Increased REM sleep tendencies were observed during the active phase while decreases were observed during rest phases. Overall effects in 24 hours are shown. These data were adapted from ambulatory recordings of $OX1R^{-/-}$ mice (Y. Y. Kisanuki, C. M. Sinton, and M. Yanagisawa, unpublished observations), $OX2R^{-/-}$ mice (8), $OX1R^{-/-};OX2R^{-/-}$ mice (10), $orexin^{-/-}$ mice (8), and humans (14). Note that the active and rest phases of laboratory mice are defined as 12 h "lights-on" and "lights-off" periods. The active (day) and rest (night) periods in the human study were defined for individual subjects, such that roughly a third of the 24 hours recordings fulfilled the criteria of rest period in each group (14). *Signifies statistically significant effects upon REM sleep amounts relative to control groups in individual studies. (*) signifies that a significant effect was observed in at least one other study of $orexin^{-/-}$ mice (1).

During the rest phase, knockout mice and narcoleptic humans (14) all exhibited a compensatory pattern of reduced REM sleep, suggesting a homeostatic response to overexpression of REM sleep during the active phase (Fig. 3). Compensatory responses were proportional to REM sleep surpluses generated in each group of subjects. However, positive REM sleep surpluses in all groups after 24-hour recordings indicated that these compensatory changes were incomplete. While overall surpluses were not consistently significant in individual studies, the pattern of magnitudes and directions of changes among narcoleptic subjects is interesting. The REM sleep patterns of $OX1R^{-/-}$ and $OX2R^{-/-}$ mice suggest that disruptions in these signaling pathways contribute independently and additively to this aspect of the narcoleptic phenotype. Notably, in a recent study of narcoleptic rats generated by expression of the *orexin/ataxin-3* transgene (by the same method used to generate mice described in Table 1), observed highly significant surpluses of REM sleep even after 24 hours (15). This effect is not simply caused by conflation of cataplexy and REM sleep as the effect remains significant even after exclusion of cataplexy-associated REM sleep from analysis in this model (C. M. Sinton and J. T. Willie, unpublished observation).

Could the above results indicate an abnormality in REM sleep homeostasis in narcolepsy-cataplexy? From the lack of significant changes in studies of narcoleptic

humans, it has been concluded that REM sleep homeostatic set-point is most likely normal (14). However, the studies of human and murine narcoleptic-cataplectic subjects to date may suffer from insufficient statistical power to detect a mild homeostatic defect in REM pressure. Alternatively, the explanation may relate to a wide normal tolerance for REM sleep surplus that is built into the homeostatic set-point mechanism. Abnormal intrusions of REM sleep during wakeful behavior in narcolepsy reduce homeostatic pressure for REM sleep during the subsequent rest phase. While experimental sleep deprivation and rebound protocols test the homeostatic tolerance for sleep *deficits*, experimental methods for defining the tolerance of REM sleep *surpluses* under various conditions in normal and narcoleptic subjects are needed to examine this further.

From a genetic perspective, loss of function in orexin signaling results in inability to gate the onset of REM sleep appropriately. By implication, orexin functions in normal animals during periods of arousal to inhibit REM sleep and its various components (such as atonia). This function may be important as the waking and REM sleep states are neurophysiologically similar in many respects. It is generally agreed that orexin cells must be maximally active during wakefulness (wake-on), especially active wakefulness associated with motor activity, and they have reduced activity during NREM sleep (16,17). While the evidence from narcoleptic mice predicts that orexin neurons are predominantly inactive during REM sleep (REM-off), conflicting reports that detected changes in immediate early gene transcription in orexin neurons or extracellular microdialysis of orexin peptides from projection fields have implied that orexin neurons remain REM-off (17) or, alternatively, could become reactivated (REM-on) (16) under certain experimental conditions. As these methods are indirect and of limited temporal resolution, direct extracellular recordings in freely-moving animals are needed.

Wake-on/REM-on neurons and wake-off/REM-on neurons of the pontine laterodorsal tegmentum (LDT) participate in the generation of REM sleep (7). These are inhibited during wakefulness and early NREM sleep by wake-on/REM-off aminergic neurons of the dorsal raphe (DR), locus coeruleus (LC) (which also modulates muscle tone), and tuberomammillary (TM) hypothalamic nucleus (which also plays a critical role in forebrain arousal (18)). These cell populations all receive orexinergic innervation and express orexin receptors, and excitatory actions of orexins have generally been observed in these regions. Thus, orexin receptor knockout mice have been used to investigate the differential roles of each receptor in LDT, DR, LC, and TM neurons in brain slices (8, 19, 20). In mice lacking both OX1R and OX2R, normal calcium transients stimulated by the nonselective orexin receptor agonist orexin-A were abolished in LDT and DR slices, demonstrating that one or both of these receptors were required (20). In mice lacking OX2R, orexin-A-induced calcium transients could not be elicited from TM slices (8), but they could be elicited in LDT, DR, and LC neurons to a similar extent as in wildtype mice (19, 20). In contrast, no calcium transients were elicited by orexin-A in slices of LDT and DR from $OX1R^{-/-}$ mice, and these results were confirmed by whole cell recording in LDT slices (20). Surprisingly, increased calcium transients were elicited in a small minority of LC neurons from $OX1R^{-/-}$ mice, suggesting that OX2R might also play a role in this region (19). Together, these results indicate that actions of orexin in TM and a small population of LC neurons are mediated by OX2R while actions of orexin in LDT, DR, and the

majority of LC neurons are mediated by OX1R. Thus, in normal animals, OX1R and OX2R would both be expected to contribute to REM sleep gating. These results are consistent with the behavioral phenotypes of orexin receptor knockout mice indicating distinct contributions from dysfunction of each orexin receptor-mediated pathway in the narcolepsy-cataplexy phenotype (Fig. 1).

III. Probing the Nature of Sleepiness in Murine Narcolepsy-Cataplexy

Following the discovery of REM sleep and its implication in the narcoleptic sleep attack, an early neurobiological model for the excessive daytime sleepiness of narcolepsy-cataplexy hypothesized an abnormal homeostatic need for REM sleep. This notion was discarded in its simplest form since sleep attacks in human narcolepsy need not have the polysomnographic features of REM sleep, and sleep monitoring failed to demonstrate a significant elevation of total REM sleep time over the entire 24-hour day (14). As recently emphasized by Mochizuki et al. (13), several other neurobiologic models have been hypothesized to account for the sleepiness of narcolepsy. Data from mice have now been brought to bear on such models, as reviewed below.

A. Arousal Defects

First, sleepiness may be the consequence of inadequate activation of fundamental arousal regions. This is consistent with heavy innervation and excitation of wake-promotingbrain regions such as aminergic and cholinergic neurons of the brainstem, hypothalamus, and basal forebrain by orexin neurons. Additionally, orexin neurons may play a more direct role in arousal as they innervate the thalamus and prefrontal cortex. Thus, the frequent transitions from wake into NREM sleep that are observed in narcolepsy-cataplexy could result from insufficient activation with reduced or unstable activity in a potentially wide array of brain regions along the arousal neuroaxis.

At baseline, OX2R- and orexin-deficient mice demonstrate mild deficits of wake amounts and increased sleep during the early active phase, although as mentioned above, the changes are not significant over 24 hours (8). Notably, reduced power in the hippocampal theta band has been detected during nocturnal wake in $orexin^{-/-}$ mice, even after treatment with modafinil (21). This may indicate a deficit in the engagement of attentional processing during periods of normal alertness.

The integrity of fundamental arousal mechanisms thought to be downstream of orexin signaling has been examined by exposure of animals to a variety of arousing stimuli. In particular, introduction of rodents to a novel environment such as a large arena or fresh bedding provide emotive stimuli that increase wakefulness and locomotor activity (1,8,13). This response depends in part upon noradrenergic (LC) and histaminergic (TM) arousal systems, although cholinergic mechanisms may participate as well. In narcoleptic mice, exposure to novelty also reliably elicits cataplectic episodes associated with direct transitions to REM sleep (8). Nevertheless, normal NREM sleep latencies and amounts of wakefulness were recorded from $orexin^{-/-}$ mice exposed to a

novel environment, suggesting that recruitment of fundamental arousal mechanisms are grossly intact and can be recruited independently of orexin under these conditions (13).

Another arousing stimulus in rodents is that of acute food restriction. Indeed, orexin neurons are directly sensitive to metabolic cues (22). Under certain conditions, mammals respond to reduced food availability by becoming more wakeful and active, and exhibit increases in activity of orexin neurons. Orexin neuron-ablated mice, however, exhibit a deficiency in this fasting-induced arousal (22).

Narcoleptic mice also respond abnormally to other stressful conditions. Orexin mice have low basal blood pressures and exhibit an attenuated sympathetic defense ("fight-or-flight") response in a resident-intruder paradigm (23), and they show reduced psychomotor activity after naloxone-induced morphine withdrawal (24). However, the effects of these stimuli upon sleep-wake states were not measured, and the specific arousal mechanisms involved require further delineation.

Overall, these studies suggest that narcolepsy is associated with failure to achieve appropriate levels and quality of arousal under various conditions. These deficits may be particularly exacerbated under special circumstances requiring an adaptive response, such as under food restriction or other stressors. Although the mechanisms of these effects remain unclear, the ability of narcoleptic mice to recruit fundamental aminergic arousal systems may be functionally intact. Extracellular recordings from freely moving orexin-deficient animals under various conditions are needed to examine the function of components of the arousal neuroaxis.

B. Circadian Control of Sleep and Wake

Second, sleepiness and fragmented sleep in narcolepsy may be caused by impaired circadian control of sleep and wake. Circadian rhythms generated by the central pacemaker in the suprachiasmatic nucleus (SCN) control the timing of wake and REM sleep (25,26), perhaps in part via projections to orexin neurons that then relay this information to sleep-regulatory and wake-regulatory regions (27). It has been hypothesized that daytime sleepiness and fragmented sleep of human narcolepsy could be caused by impaired circadian control since circadian signals help time and consolidate sleep-wake behavior (25,28,29).

Orexin$^{-/-}$, *OX2R*$^{-/-}$, and orexin neuron-ablated mice do not exhibit overtly abnormal timing of sleep and wake when housed under conditions of a normal light-dark (12 hours on, 12 hours off) cycle (1,2,8,13). Notably, however, the amplitudes of wakefulness (1,2,8,13) and of running wheel activity (M. Mieda, personal communication, 2005) are reduced in narcoleptic animals during the dark phase of the cycle. As light itself has a direct suppressive effect on locomotor activity and wakefulness in nocturnal rodents, free-running rhythms of *orexin*$^{-/-}$ mice were also examined under conditions of constant darkness. After habituation to constant darkness, *orexin*$^{-/-}$ mice maintain a normal periodicity of free-running body temperature rhythms (13) and wheel running behavior (M. Mieda, personal communication, 2004).

Likewise, chronic habituation of wildtype mice to a "food shift" paradigm in which food availability is restricted to a few hours at the same time each day induces a predictable circadian pattern of food-anticipatory wakefulness and locomotor activity, even in SCN-lesioned animals (30). This phenomenon is evidence for existence of a food-entrainable oscillator that is functionally independent of the master

circadian oscillator residing in the SCN. Remarkably, orexin neuron-ablated mice exhibit normal timing but attenuated amounts of wakefulness and locomotor activity during the food shift paradigm regardless of light or dark conditions (31). Despite this deficit of food-anticipatory arousal, the narcoleptic mice have normal levels of wakefulness and food consumption during the subsequent consummative phase when food is presented, and maintain body weights in a manner similar to controls over the course of the experiment.

While orexin may modulate the effectiveness of the master circadian and food-entrainable oscillators, the intrinsic periodicity of these pacemakers appears to be intact. Reduced activation of fundamental arousal systems in response to the circadian signal may underlie reduced amplitudes of active behavior, but fragmented sleep-wake behavior in narcoleptic mice is unlikely to result directly from abnormal circadian control.

C. Homeostatic Mechanisms of NREM Sleep

Third, it has been hypothesized that sleepiness results from abnormal NREM sleep homeostasis in narcolepsy, with an inappropriately rapid accumulation or intense expression of sleep pressure (32). Two studies have tested the ability of narcoleptic-cataplectic mice to respond to challenges to sleep-wake homeostasis. Traditionally, examination of sleep recovery following sleep deprivation (by gentle handling in rodents) allows examination of such mechanisms. Compared to wildtype mice, $orexin^{-/-}$ mice demonstrated a normal dose-dependent response to acute total sleep deprivation (2, 4, and 8 hours) with normal decreases in NREM sleep latencies, an increase in NREM EEG delta power, and subsequent recovery of NREM sleep deficits at a normal rate and to a normal degree (13). After eight hours of deprivation, however, $orexin^{-/-}$ mice appeared to express REM sleep pressure more acutely than wildtypes during rebound, but the overall effect of sleep deprivation on REM sleep homeostasis is difficult to interpret in this study as REM sleep occurring directly after wakefulness was defined operationally as cataplexy (by electroencephalographic rather than behavioral criteria) and excluded from total REM sleep amounts (13). Nevertheless, the authors concluded that across a variety of measures, $orexin^{-/-}$ mice appear to have essentially normal homeostatic responses to sleep deprivation.

The food shift paradigm described in the preceding section, is also a complex homeostatic challenge that results in new lower body weight set point and chronic changes in arousal patterns over 24 hours in wildtype mice (31). When food was restricted to the day, the wildtype group maintained a normal mean level of wakefulness and sleep over 24 hours, but the narcoleptic group stabilized at a significantly lower amount of daily wakefulness despite reductions in food intake and body weight that were identical to those of the wildtype group. In contrast, when food was restricted to the night, wildtype mice achieved a significant elevation in wakefulness while narcoleptic mice did not. These findings imply a limited ability of narcoleptic mice to readjust homeostatic sleep-wake set-points in response to this particular challenge. Thus, while orexin may not play a fundamental role in sleep-wake homeostasis following sleep deprivation and recovery, orexin does modulate sleep-wake homeostasis in response to complex metabolic demands. Perhaps orexin-dependent increases in

wakefulness provide an adaptive advantage in a natural environment by increasing the opportunity to encounter food in times of scarcity.

D. Behavioral State Instability

Finally, a fourth model of sleepiness in narcolepsy has been suggested in which sleepiness results not from an arousal deficit per se, but from uncoordinated activity in sleep-wake systems resulting in generalized behavioral state instability in the absence of orexin (13). This model emerges in part from an analogy comparing the composite mechanisms controlling sleep-wake transitions to a bistable switch (33). Wake-promoting neuronal substrates in the posterior hypothalamus and brainstem interact with sleep-promoting substrates in the anterior hypothalamus in a mutually inhibitory relationship to create tension between processes generating sleep and wake states. This system is in turn modulated by orexin which stabilizes the "sleep switch" and opposes sleep propensity by stabilizing ongoing wakefulness. Increased orexinergic tone (as occurs normally during the active phase) promotes arousal and inhibits transitions among states. Decreased orexinergic tone (as would occur during a rest phase) allows expression of underlying homeostatic drive for sleep, and a rapid transition to sleep can appropriately occur. Complete lack of orexin, however, creates instability in the system and unregulated expression of otherwise normal homeostatic drives. This results in rapid unregulated transitions between states and/or mixed behavioral states. This model is attractive in that it is consistent with the observation that *orexin*$^{-/-}$ mice have more transitions between all behavioral states (13) (Fig. 2) and that wakefulness and sleep are fragmented throughout both the rest and active phases (8,13). Nevertheless, this model will require further experimental scrutiny.

Notably, mice deficient in histamine (histidine decarboxylase knockout mice) also exhibit severe behavioral state instability, with elevated episode frequencies of all states and reduced durations of wake and NREM (18). Unlike narcoleptic animals, however, they do not exhibit cataplexy or direct transitions to REM sleep. As activation of histaminergic neurons of the TM depends upon OX2R (8), unstable activation of histaminergic neurons of the TM might therefore provide part of the mechanism for behavioral state instability and sleepiness in *OX2R*$^{-/-}$ and *orexin*$^{-/-}$ mice.

IV. Orexin to the "Rescue": Therapy for Narcolepsy-Cataplexy

In narcoleptic patients, excessive sleepiness is currently treated using amphetamines, modafinil, or gamma-hydroxybutyrate (sodium oxybate), while cataplexy is relieved by tricyclic antidepressants, norepinephrine reuptake inhibitors, or gamma-hydroxybutyrate. These drugs can be problematic due to limited effectiveness, undesirable side effects (such as insomnia, symptom rebounds, and cardiovascular complications), and the potential for abuse. Since human narcolepsy-cataplexy may result from selective degeneration of orexin neurons, replacement therapies based on administration of orexin receptor agonists should target the fundamental pathophysiology of the disorder. Notably, intracerebroventricular injections of orexin peptides, administered acutely in wildtype rodents, have been shown to increase wakefulness and suppress both non-REM and REM sleep (34).

By two distinct methods of replacing orexin peptides in the *orexin/ataxin-3* model of narcolepsy-cataplexy, rescue of symptoms has been demonstrated (35). Chronic over-production of orexin-A and orexin-B from an ectopically expressed *prepro-orexin* transgene (*CAG/orexin;orexin/ataxin-3* double transgenic mouse, Table 1) prevented the development of narcolepsy-cataplexy symptoms in the absence of endogenous orexin neurons (Fig. 4). Similarly, intracerebroventricular bolus administrations of orexin-A acutely increased wakefulness, suppressed sleep, and inhibited cataplectic attacks in narcoleptic mice (35). Indeed, orexin-A more effectively increased wakefulness in *orexin/ataxin-3* transgenic mice than in wildtype controls. Together, these findings provide strong evidence of the specific causal relationship between absence of orexin peptides in the brain and the development of the narcolepsy-cataplexy syndrome. The success of pharmacological experiments suggests that the neural mechanisms required for orexin-mediated arousal and suppression of cataplexy (orexin receptors, intracellular signaling, postsynaptic neural networks, and other downstream neurotransmitter pathways) remain anatomically and functionally intact.

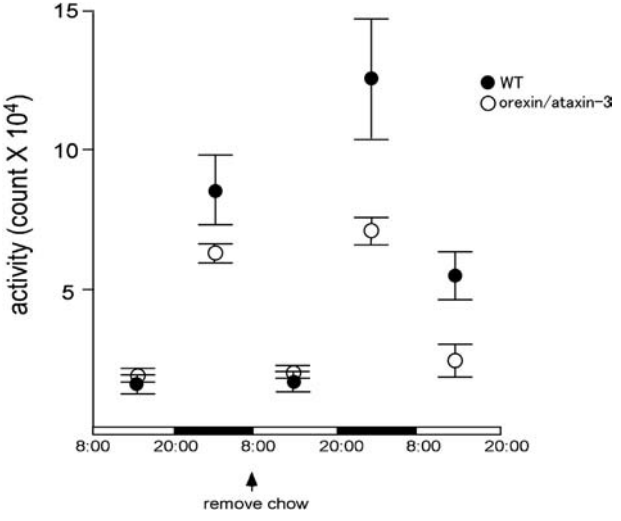


Figure 4 Genetic rescue of narcolepsy-cataplexy in orexin neuron-ablated mice. Illustrative hypnograms showing sleep-wake cycles of typical transgenic and wildtype mice. Hypnograms represent concatenated 20-second epochs of EEG/EMG activity, scored as wake (W), NREM sleep (N), or REM sleep (R). Seven hours per mouse, including transitions from light phase to dark phase (*solid bar*), are shown. The *orexin/ataxin-3* transgenic mouse exhibits fragmentation of wakefulness during the dark phase and abnormal periods of REM sleep that occur immediately after wakefulness (*arrowheads*) or after <60 seconds of preceding NREM sleep (*arrows*). In contrast, the *CAG/orexin;orexin/ataxin-3* double transgenic mouse has more consolidated wakefulness during the dark phase. As in the wild type mouse, no direct or premature transitions from wake to REM sleep were observed in the double transgenic mouse. *Source*: Adapted from Ref. 35.

Notably, orexin-A administrations to narcoleptic mice did not result in sharp rebounds of sleep (35). When administered in coordination with the active phase, the orexin-induced gains in cumulative wakefulness lasted as long as 24 hours after injection in narcoleptic mice. The absence of such rebounds, a potentially confounding factor in therapies for excessive daytime sleepiness based on classical psychostimulants, suggests that orexin-based therapies may more safely maintain wakefulness. Interestingly, recent studies hint that the orexins may promote attention and memory processes (36,37); and *orexin*$^{-/-}$ mice have subtle electrophysiological signs of decreased attentional processes during wakefulness, even after modafinil administration (21). Orexin-based therapies may therefore also improve cognitive function in coordination with increased arousal.

Utilizing OX2R-deficient narcoleptic Dobermans, John et al. have suggested that large peripheral doses of orexin-A may reverse sleep/wake fragmentation and cataplexy in some dogs (38). This suggests a role for OX1R signaling in reducing cataplexy and normalizing sleep patterns. However, Fujiki et al. failed to duplicate these results, even at higher doses (39); and the efficacy of peripheral administrations of orexin peptides remains unclear.

The demonstration of a genetic rescue of narcolepsy-cataplexy may have theoretical implications for future human therapies, which might involve orexin gene therapy utilizing viral vectors, or transplantation of orexin neurons or stem cell precursors. The effects of chronic orexin administrations upon behavioral state transitions in animals and the effects of small-molecule, orally-active orexin receptor agonists in humans clearly merit further investigation.

V. Conclusion: A Molecular Genetic Model of the Narcolepsy-Cataplexy Phenotype

While the cause of orexinergic-specific neuronal degeneration in human narcoleptics remains a mystery, animal models have given us valuable clues as to the role of orexin in the disorder. Genetically modified animals have also shed light upon the underlying mechanisms of the syndrome. From these studies, a molecular genetic model of the function of the orexin neuropeptide system during wakefulness and the symptoms of the narcolepsy-cataplexy phenotype is proposed (Fig. 5). The similar inability to maintain wakefulness that is observed in orexin-deficient mice and in OX2R-deficient mice implicates absence of signaling through this receptor in the sleepiness and proposed behavioral instability of narcoleptic mice. However, both OX1R and OX2R pathways must play independent, mutually compensating roles in the gating of REM sleep, as evidenced in both *OX1R*$^{-/-}$ and *OX2R*$^{-/-}$ mice by decreases in REM sleep latencies, and in *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ and orexin-deficient mice by direct transitions to REM sleep and significant increases in nocturnal REM sleep. Likewise, the mild cataplexy phenotype of *OX2R*$^{-/-}$ mice compared to *OX1R*$^{-/-}$;*OX2R*$^{-/-}$ and orexin-deficient mice implies an important modifying influence of OX1R signaling upon triggering of cataplexy. This may occur through a direct effect upon the brainstem muscle tone inhibitory system (although *OX1R*$^{-/-}$ mice do not exhibit cataplexy), or through indirect effects upon the pontine REM sleep generator or through other indirect limbic effects.

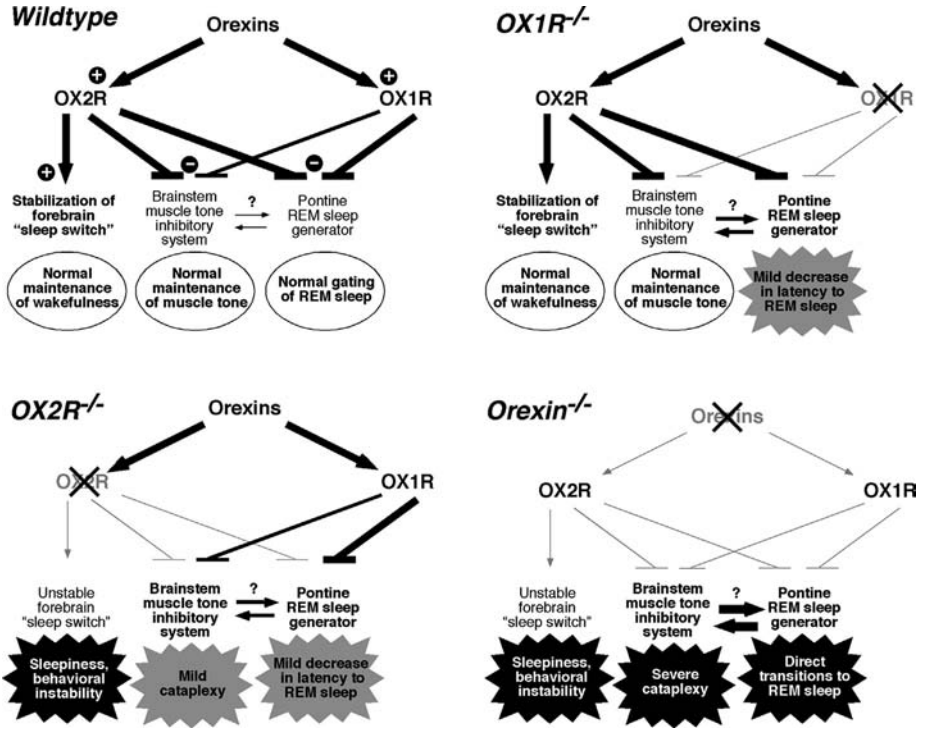


Figure 5 Proposed genetic model of orexin neuropeptide system in behavioral regulation and narcolepsy-cataplexy. The proposed genetic relationships among orexin signaling pathways extend a previous model (8). Comparisons across wildtype, $OX1R^{-/-}$, $OX2R^{-/-}$, $OX1R^{-/-}$; $OX2R^{-/-}$ (not shown), and $orexin^{-/-}$ mice suggest that OX2R signaling has a predominant role in the maintenance of wakefulness since $OX2R^{-/-}$, $OX1R^{-/-}$;$OX2R^{-/-}$, and $orexin^{-/-}$ mice all exhibit similar signs of sleepiness while $OX1R^{-/-}$ mice do not. Similar deficits in REM sleep latency in $OX1R^{-/-}$ and $OX2R^{-/-}$ indicate that both receptors make important contributions to the gating of REM sleep. Absence of signaling through both pathways in $OX1R^{-/-}$;$OX2R^{-/-}$ and $orexin^{-/-}$ mice results in frequent direct transitions to REM sleep. The role of OX1R in stabilization of muscle tone is less clear: $OX1R^{-/-}$ mice do not exhibit cataplexy, yet functional OX1R signaling provides partial protection against cataplexy in $OX2R^{-/-}$ mice. Arrows with question mark shown between the muscle tone inhibitory system and the REM sleep generator invoke a mechanism of positive feedback to explain the rapid transition to REM sleep that occurs after cataplexy is triggered in mice. Lines with arrows indicate facilitation, flat-headed lines indicate disfacilitation. Thickness of lines indicate signaling input. X-marks signify dysfunction in genetic pathways. Circled and exploded text captions represent observed behavioral features in each genotype.

The genetic and pharmacologic rescue of the narcolepsy-cataplexy phenotype gives hope that orexin receptor agonists will one day provide improved therapy for the human disorder. Because cataplexy and sleep attacks can be discriminated by simple objective behavioral criteria or by polysomnographic measures,

narcoleptic-cataplectic mice are well suited for further use in the pharmaceutical discovery process.

VI. Summary

Recent work with animal models of narcolepsy-cataplexy has critically advanced the understanding of the pathophysiology and the potential treatment of this debilitating disorder. *Prepro-orexin* knockout mice provided the earliest evidence that deficiency of orexin (hypocretin) neuropeptides causes narcolepsy-cataplexy. Systematic comparisons of behavioral, pharmacological, and polysomnographic phenotypes of a variety of genetically modified mice provide a unique perspective on the fundamental abnormalities of the disorder. Additionally, intracellular calcium imaging and whole-cell recording of brain tissue from orexin receptor-deficient rodents verifies the distinct roles of orexin signaling pathways in the neurobiology of behavioral states. Together, these studies enhance our understanding of the orexin system in normal sleep-wake regulation and pathological conditions. Finally, genetic and pharmacological rescue experiments in orexin neuron-ablated mice provide strong indications that orexin receptors will be invaluable therapeutic targets for narcolepsy-cataplexy.

Acknowledgments

J.T.W. received funding through fellowships from the Medical Scientist Training Program of The University of Texas Southwestern Medical Center and the Merck Foundation. M.Y. is an Investigator of the Howard Hughes Medical Institute. The authors thank C. M. Sinton, T. E. Scammell, M. Mieda, Y. Y. Kisanuki, and S. Nishino for intellectual contributions. This work was also supported in part by research funds from the Keck Foundation, the Perot Family Foundation, and the ERATO/JST. This work is dedicated to S. B. Willie.

References

1. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammel T, Lee C, Richarson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagiasawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–51.
2. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001; 30:345–54.
3. Nishino S, Riehl J, Hong J, Kwan M, Reid M, Mignot E. Is narcolepsy a REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. Neuroscience Research 2000; 38:437–46.
4. John J, Wu MF, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 2004; 42:619–34.
5. Baker TL, Foutz AS, McNerney V, Mitler MM, Dement WC. Canine model of narcolepsy: genetic and developmental determinants. Exp Neurol 1982; 75:729–42.
6. Ripley B, Fujiki N, Okura M, Mignot E, Nishino S. Hypocretin levels in sporadic and familial cases of canine narcolepsy. Neurobiol Dis 2001; 8:525–34.

7. Hishikawa Y, Shimizu T. Physiology of REM sleep, cataplexy, and sleep paralysis. Adv Neurol 1995; 67:245–71.
8. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, Elmquist JK, Kohlmeier KA, Leonard CS, Richardson JA, Hammer RE, Yanigasawa M. Distinct narcolepsy syndromes in orexin receptor type 2 (OX2R) and orexin knockout mice: Molecular Genetic dissection of non-REM and REM sleep regulatory processes. Neuron, 2003.
9. Kisanuki YY, Chemelli RM, Sinton CM, Williams SC, Richardson JA, Hammer RE, Yanagisawa M. The Role of Orexin Receptor Type-1 (OX1R) in the Regulation of Sleep. Sleep 2000; 23:A91.
10. Kisanuki YY, Chemelli RM, Tokita S, Willie JT, Sinton CM, Yanagisawa M. Behavioral and polysomnographic characterization of orexin-1 receptor and orexin-2 receptor double knockout mice. Sleep 2001; 24:A22.
11. Schenck CH, Mahowald MW. Motor dyscontrol in narcolepsy: rapid-eye-movement (REM) sleep without atonia and REM sleep behavior disorder. Annals of Neurology 1992; 32:3–10.
12. Kiyashchenko LI, Mileykovskiy BY, Lai YY, Siegel JM. Increased and decreased muscle tone with orexin (hypocretin) microinjections in the locus coeruleus and pontine inhibitory area. J Neurophysiol 2001; 85:2008–16.
13. Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knock-out mice. J Neurosci 2004; 24:6291–300.
14. Broughton R, Dunham W, Newman J, Lutley K, Duschesne P, Rivers M. Ambulatory 24 hour sleep-wake monitoring in narcolepsy-cataplexy compared to matched controls. Electroencephalogr Clin Neurophysiol 1988; 70:473–81.
15. Beuckmann CT, Sinton CM, Williams SC, Richardson JA, Hammer RE, Sakurai T, Yanagisawa M. Expression of a poly-glutamine-ataxin-3 transgene in orexin neurons induces narcolepsy-cataplexy in the rat. J Neurosci 2004; 24:4469–77.
16. Kiyashchenko LI, Mileykovskiy BY, Maidment N, Lam HA, Wu MF, John J, Peever J, Siegel JM. Release of hypocretin (orexin) during waking and sleep states. J Neurosci 2002; 22:5282–6.
17. Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammel TE. Fos expression in orexin neurons varies with behavioral state. J Neurosci 2001; 21:1656–62.
18. Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS. Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. Journal of Neuroscience 2002; 22:7695–711.
19. Kohlmeier KA, Chemelli RM, Willie JT, Kisanuki YY, Yanagisawa M, Leonard CS. Hypocretin/Orexin (H/O) elevates [Ca2+]i in locus ceruleus (LC) neurons via activation of OX1 and OX2 receptors. Sleep 2003; 26:A21–22.
20. Tyler CJ, Kohlmeier KA, Willie JT, et al. Orexin receptor-1 mediates multiple hypocretin/orexin (H/O) actions in laterodorsal tegmentum (LDT) and dorsal raphe (DR), Neuroscience 2002, Orlando, Florida, 2002. Vol. Abstract Viewer/Itinerary. Washington DC: Society for Neuroscience.
21. Willie JT, Renthal W, Chemelli RM, Miller MS, Scammel TE, Yanagisawa M, Sinton CM. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience 2005; 130:983–95.
22. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, Yanagisawa M, Sakurai T. Hypothalamic Orexin Neurons Regulate Arousal According to Energy Balance in Mice. Neuron, 2003.
23. Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, Fukuda Y, Kuwaki T. Attenuated defense response and low basal blood pressure in orexin knockout mice. American Journal of Physiology - Regulatory Integrative & Comparative Physiology 2003; 285:R581–93.
24. Georgescu D, Zachariou V, Barrot M, Mieda M, Willie JT, Eisch AJ, Yanagisawa M, Nestler EJ, DiLeone RJ. Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. Journal of Neuroscience 2003; 23:3106–11.
25. Edgar DM, Dement WC, Fuller CA. Effect of SCN lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. J Neurosci 1993; 13:1065–79.
26. Edgar DM, Seidel WF. Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. J Pharmacol Exp Ther 1997; 283:757–69.
27. Abrahamson EE, Leak RK, Moore RY. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. Neuroreport 2001; 12:435–40.
28. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Research Online 1998; 1:159–65.

29. Wurts SW, Edgar DM. Circadian and homeostatic control of rapid eye movement (REM) sleep: promotion of REM tendency by the suprachiasmatic nucleus. Journal of Neuroscience 2000; 20:4300–10.
30. Stephan FK. The "other" circadian system: food as a Zeitgeber. Journal of Biological Rhythms 2002; 17:284–92.
31. Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M. Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. J Neurosci 2004; 24:10493–501.
32. Besset A, Tafti M, Nobile L, Billiard M. Homeostasis and narcolepsy. Sleep 1994; 17:S29–34.
33. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24:726–31.
34. Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Poster RFA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci USA 1999; 96:10911–6.
35. Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci USA 2004; 101:4649–54.
36. Jaeger LB, Farr SA, Banks WA, Morley JE. Effects of orexin-A on memory processing. Peptides 2002; 23:1683–8.
37. Wu M, Zhang Z, Leranth C, Xu C, van den Pol AN, Alreja M. Hypocretin increases impulse flow in the septohippocampal GABAergic pathway: implications for arousal via a mechanism of hippocampal disinhibition. J Neurosci 2002; 22:7754–65.
38. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Res Online 2000; 3:23–8.
39. Fujiki N, Yoshida Y, Ripley B, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin-ligand-deficient narcoleptic dog. Sleep 2003; 26:953–9.
40. Aldrich MS. Diagnostic aspects of narcolepsy. Neurology 1998; 50:S2–7.
41. Zorick FJ, Salis PJ, Roth T, Kramer M. Narcolepsy and automatic behavior: a case report. J Clin Psychiatry 1979; 40:194–7.

26
Hypocretin-1 Studies in Cerebrospinal Fluid: European Experience

CHRISTIAN R. BAUMANN and CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

SEBASTIAAN OVEREEM
Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen and Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

GERT JAN LAMMERS
Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

I. Introduction

After the discovery of a hypocretin-2 receptor gene mutation in canine narcolepsy and the observation of a narcolepsy-like phenotype in prepro-hypocretin gene knock-out mice, a disturbed hypocretin transmission was searched also in human narcolepsy. Using a radioimmuno assay (RIA) the Stanford group in January 2000 first described the absence of the peptide hypocretin-1 in the cerebrospinal fluid (CSF) of seven out of nine patients with narcolepsy from Leiden (Holland) (1). This result was confirmed in a larger group of patients from Stanford, Hattiesburg, Atlanta (US), Leiden, Zurich (Switzerland), Prague (Czech Republic) and Trondheim (Norway), again tested in Stanford (2), as well as in a third group of patients from Zürich and Montpellier (France), this time tested in Zurich (3). Measurements of CSF hypocretin-2 levels in human narcolepsy were reported only once in the literature but remained, so far, unconfirmed (4).

This chapter will focus on European (and worldwide) experience in methodological and clinical aspects of CSF hypocretin-1 measurements.

II. Determination of CSF Hypocretin-1 Levels

Soon after the report of low/undetectable CSF hypocretin-1 levels in narcoleptics from Stanford, few centres in Northern America, Europe and Japan started to perform measurements with a commercially available radioimmuno assay (RIA) kit (from Phoenix Pharmaceuticals, Mountain View, CA, USA). As the assessment of CSF hypocretin-1 levels is associated with methodological pitfalls (see below) (5), some of these centres subsequently abandoned these measurements.

The first studies used a protein-extraction procedure prior to the hypocretin RIA. Later studies showed that the measurements were just as reliable in crude, unextracted CSF (6). However, variability decreases after the extraction step, and detection limits may be lower. The "crude" method is much simpler to perform and requires a volume of only 200 μl for duplicate measures, compared to 1 ml for the extracted assay.

Hypocretin-1 is a stable peptide (7). Storage at room temperature for one week does not affect hypocretin levels, neither does repeated freezing and thawing. Also storage at $-70^{\circ}C$ for >10 years seems not to affect the concentration. Neither age, medication, nor the time of day at which the lumbar puncture is performed, have a significant influence on the interpretation of the results, although diurnal changes in physiological CSF hypocretin concentrations probably exist (8,9).

The interassay variability of the Phoenix RIA kit is high (interassay coefficient of variation up to 25%). This necessitates the use of standardized protocols with reference CSF at different concentrations. Determination of CSF hypocretin-1 levels in control groups is necessary to calculate normal values. Due to the high interassay variability and the use of different reference CSF samples, hypocretin-1 levels and therefore the definition of pathologically low hypocretin-1 levels may vary between different laboratories (5). In Leiden and Zurich, reference samples based on pooled human CSF are used. These samples were, at one point, "cross-referenced" with Stanford [blinded measurements of same CSF samples in both laboratories (5)].

The detection limit of the Phoenix RIA changes from lab to lab, and from assay to assay. For crude CSF, it typically is around 40–80 pg/ml, meaning that values below this limit should be regarded as "undetectable", and specific concentrations should not be mentioned. In a lot of studies, the detection limit is not mentioned at all, and calculations are made with numbers below the detection limit.

In conclusion, the comparison of hypocretin-1 values from different assays requires the use of a standard reference samples in different concentrations. Furthermore, only levels above the detection limit of that particular assay should be considered. Finally, when precise comparisons are needed (e.g., CSF hypocretin-1 levels before and after treatment in a single patient), samples should be run in a single assay. Hopefully a consensus on the methodology to be used, including the need for standardized reference samples to be shared in different laboratories, will soon be available (see workshop report on CSF measurements in this book).

III. CSF Hypocretin-1 in Narcolepsy and Other Disorders

A. Narcolepsy

The high sensitivity of low/undetectable CSF hypocretin-1 levels for narcolepsy-cataplexy, first reported in Stanford and Leiden (1,2,6), has been confirmed in other patients" series from Europe (3), Japan (10) and the USA (11). A recent review of 174 narcoleptics reported in the literature found a sensitivity of 89% of low or undetectable levels of CSF hypocretin-1 levels (12). In most of these studies, the presence of definite ("typical," "true," "clear-cut") cataplexy was considered the "golden standard" for the diagnosis of narcolepsy. Definite cataplexy consists of bilateral muscle weakness triggered in particular by laughter, and lasts less than several minutes with a

preserved consciousness. Several studies have in fact shown that narcolepsy with atypical or without cataplexy usually is associated with normal CSF hypocretin-1 levels (2,3,6,10,12,13). However, narcolepsy with definite cataplexy and normal CSF hypocretin-1 levels does also exist. Most of these patients are HLA negative, familial or symptomatic cases of narcolepsy with cataplexy (2,3,6,12–14). These observations suggest the existence of narcolepsy phenotypes without any demonstrable defect in the hypocretin neurotransmission.

The experience in Leiden reflects these general rules. When CSF hypocretin-l levels are measured in patients with definite cataplexy, who are HLA-positive and have no family history, the sensitivity of undetectable hypocretin levels is almost 100 % (Fig. 1). Similarly, in Zurich, only one out of 19 consecutive patients with sporadic narcolepsy and definite cataplexy had normal CSF hypocretin-l levels (Fig. 2). A HLA-DQB1*0602-positive monozygotic twin pair concordant for narcolepsy with cataplexy had normal hypocretin levels (14).

Current knowledge suggests that clinical manifestations of narcolepsy occur once CSF hypocretin levels are already low or undetectable. In one prepubertal child CSF hypocretin levels were found to be low as early as three weeks after the onset of clinical symptoms, which in turn preceded the appearance of sleep onset REM periods (15).

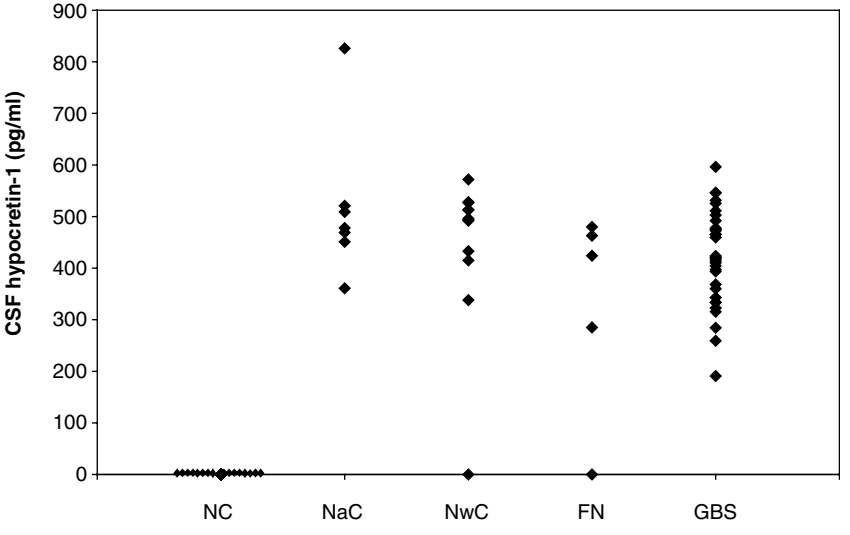


Figure 1 Hypocretin-1 levels in crude CSF of patients from the Leiden Narcolepsy Clinic (n = 74). The detection limit of the assay was 70 pg/ml. Leiden patients who were measured in Stanford were published before (1,2), and are not represented here, neither are measurements for other centres in the Netherlands or Europe Familial narcolepsy was defined as the presence of at least one family member with cataplexy and/or at least two family members with excessive daytime sleepiness. For the definition of typical cataplexy, see text. Atypical cataplexy was defined as sudden episodes of weakness triggered by emotions who do not fullfill the criteria of typical cataplexy. NC = narcolepsy-cataplexy (n = 17), NaC = narcolepsy, atypical cataplexy (n = 7), NwC = narcolepsy without cataplexy (n = 12), FN = familial narcolepsy (n = 5), GBS = Guillain-Barré syndrome (n = 33).

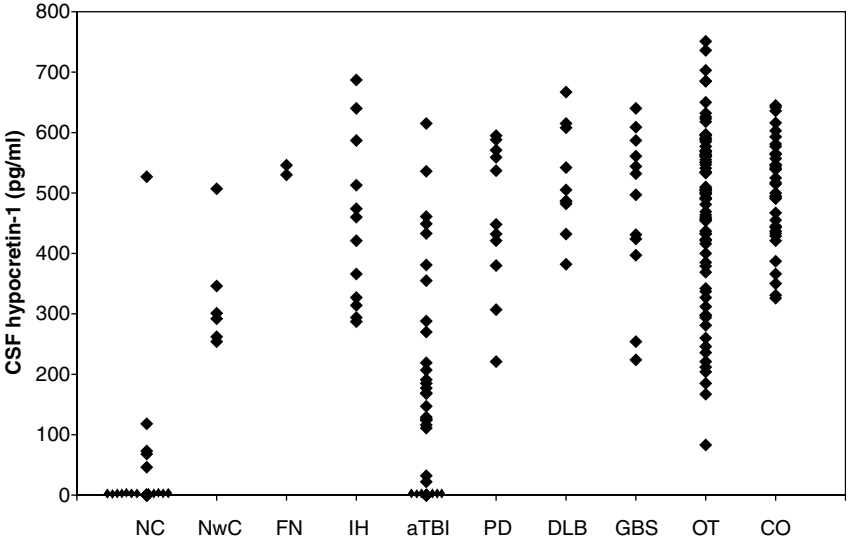


Figure 2 Hypocretin-1 levels in the crude CSF of Zurich patients with a variety of sleep and neurologic disorders and in controls (n = 220). CSF levels below 320 pg/ml are considered low (mean of control values −2SD), the detection limit is 20 pg/ml. NC = narcolepsy-cataplexy (n = 17), NwC = narcolepsy without cataplexy (n = 6), FN = familial narcolepsy (n = 2), IH = idiopathic hypersomnia (n = 12), aTBI = acute traumatic brain injury (n = 35), PD = Parkinson's disease (n = 11), DLB = dementia with Lewy bodies (n = 10), GBS = Guillain-Barré syndrome (n = 13), OT = other neurological/psychiatric disorders (n = 79), CO = control group (n = 36) without neurological disorders (CSF obtained during spinal anesthesia, age range 16–82 years).

In the first report of low/undetectable hypocretin levels in narcoleptic patients compared to controls, cut-off values were not discussed. The observation of narcoleptics with definite cataplexy and low but detectable levels of CSF hypocretin-1 [30% of all patients in recent review of 174 cases reported in the literature (12)] raised a discussion about the range of normal values and the cut-off point for the diagnosis of narcolepsy. This discussion is more complicated than expected at first sight. As mentioned before, there happens to be a large inter-assay variability. For that reason there is not just one absolute value for each RIA that can be considered as cut-off value. Stanford defines hypocretin levels <110 pg/ml as "low" (based on a Quantitative Receiver Operating Curve analysis) and levels between 110 and 200 pg/ml (artificial cut off with best specifity) as "intermediate". These values do not apply, however, for other centers. In Zurich, for example, values are considered "low" when <320 pg/ml (calculated by mean values of healthy controls minus 2 standard deviations).

The question rises whether hypocretin determinations are of any diagnostic help considering the fact that low/undetectable levels mostly occur, at least in sporadic and idiopathic narcolepsy, in the presence of definite cataplexy. Based on current knowledge CSF hypocretin-1 measurements can be recommended in patients in whom diagnosis may be difficult or questionable because of 1) prominent psychiatric

symptoms; 2) comorbidity with sleep disorders such as obstructive sleep apnea syndrome; 3) (small) children (no MSLT criteria, cataplexy difficult to assess); 4) atypical electrophysiological findings; 5) already receiving treatment (making MSLT testing unreliable). In addition, data on CSF hypocretin-1 levels in familial narcolepsy, atypical narcolepsy, narcolepsy without cataplexy, HLA-negative narcolepsy and symptomatic narcolepsy will increase our understanding about the spectrum of this disorder.

B. Neurodegenerative Disorders

Both idiopathic Parkinson's disease (IPD) and dementia with Lewy bodies (DLB) can present with narcolepsy-like symptoms such as severe excessive daytime sleepiness with "sleep attacks," hallucinations, and REM sleep behavior disorder suggesting the possibility of an involvement of the hypocretin system in these disorders

Reports regarding CSF hypocretin levels in Parkinson's disease have been controversial. First observations in single patients, in whom clinical symptoms were not specified, suggested normal levels in Parkinson's disease (2,6). Two systematic studies from Leiden and Zurich, in which patients with idiopathic Parkinson's disease and excessive daytime sleepiness (EDS) were tested, revealed normal (lumbar) CSF hypocretin-1 levels (16,17). This was also true for patients with, in addition to EDS, hallucinations, REM sleep behavior disorder, very short mean sleep latencies, and sleep onset REM periods on MSLT (Fig. 2) (17). Similarly, CSF hypocretin-1 levels were found to be normal in patients with dementia with Lewy bodies and EDS (5).

A recent study, however, reported low and undetectable levels in a series of patients with advanced Parkinson's disease (but unknown clinical symptoms) (18). In the latter study, however, hypocretin-1 measurements were performed in CSF obtained from ventricular puncture. This finding was replicated in Leiden in a Parkinson's patient in whom ventricular CSF was obtained (unpublished observation). These data suggest the possibility of a discrepancy between lumbar and ventricular hypocretin-1 assessments, an issue that requires further studies.

C. Neuroinflammatory Disorders

Considering the suspected, though unproven, autoimmune pathogenesis of narcolepsy, CSF hypocretin-1 studies in autoimmune disorders of the central nervous system (CNS) are of particular interest.

In four patients with anti-Ma2 associated encephalitis and EDS CSF hypocretin-1 levels were found to be undetectable (19). In a series of mainly Japanese patients with acute Guillain-Barré syndrome (GBS), Miller-Fisher syndrome and chronic inflammatory demyelinating polyneuropathy 24 patients had low levels and seven patients had undetectable levels (20). However, in a series of 11 Caucasian GBS patients, CSF hypocretin-1 levels were found to be normal in a majority of tested patients (Fig. 2) (21). The same was true for an extended Leiden study, in which 33 Caucasian GBS patients also had normal levels (Fig. 1). Low or undetectable CSF hypocretin-1 levels were reported also in association with acute disseminated encephalomyelitis (22,23), and multiple sclerosis (24,25).

Noteworthy, in patients with autoimmune disorders of the CNS and low/undetectable CSF hypocretin-1 levels cataplexy is usually not observed, and EDS

has been reported in only a few of these patients. (The total number of reported cases is also low). Conversely, Bassetti et al. have reported a patient with a narcoleptic tetrade resulting from an acute disseminated encephalomyelitis in the dorsal pontine tegmentum in whom CSF-hypocretin-1 levels were normal (13).

D. Other Neurologic Disorders

Low CSF hypocretin-1 levels were observed in patients with diencephalic stroke (26), hypothalamic tumor (27), and other brain disorders including infectious encephalitis, Hashimoto thyreoiditis, myotonic dystrophy, Niemann-Pick disease type C, Whipple's disease, normal pressure hydrocephalus, and central vertigo (specific references in (12)).

Of particular interest is the preliminary observation of low levels in patients with traumatic brain injury (TBI) (6). In a systematic and prospective study of 35 patients with acute TBI, Baumann et al. confirmed this finding (28). CSF hypocretin-1 levels were significantly lower in 93% of patients with a Glasgow Coma Scale ≤ 8 and in 96% of patients with posttraumatic brain CT changes when compared to normal controls (Fig. 2). In 27 patients with moderate/severe TBI, hypocretin was determined in ventricular CSF, whereas lumbar puncture was performed in 3 patients with moderate/severe TBI (in 2 of whom hypocretin was not detectable in CSF), and in 5 patients with mild TBI. This finding may reflect structural hypothalamic damage and be linked with the frequent development of sleep-wake disorders after TBI. Further studies are needed however, to rule out the possibility that this result may be related at least in parts to the ventricular origin of CSF (see above), the ventricular drainage itself (which greatly increases CSF production, and possibly dilutes the hypocretin concentration), and vigilance state per se.

IV. Summary and Conclusions

A deficient hypocretin neurotransmission has been found in animal models of narcolepsy and in human narcolepsy. In clinical practice, measurements of CSF hypocretin-1 levels appear to be the most sensitive ancillary test for diagnosing narcolepsy with cataplexy. Due to methodological issues experienced laboratories should perform determinations. A tight collaboration between different laboratories in Europe and the USA has led to a standardization of this determination procedure in these centres. Many questions remain, however, still unanswered, e.g. the definition of cut-off values for the diagnosis of narcolepsy.

In most cases of familial, HLA-negative or symptomatic narcolepsy-cataplexy with normal/detectable CSF hypocretin-1 levels, clinical symptoms and in particular the characteristics of cataplexy appear to differ from those of sporadic narcolepsy with definite cataplexy. Nevertheless, normal CSF hypocretin-1 levels can be observed in a few patients with narcolepsy and definite cataplexy. On the other hand, low/undetectable CSF hypocretin-1 levels have been reported also in neurological disorders with and without hypersomnia/excessive daytime sleepiness and cataplexy. These observations raise questions about the nature of the link between hypocretin deficiency and clinical manifestations in human narcolepsy.

Acknowledgments

CSF of GBS patients measured at Leiden University was kindly provided by P.A. van Doorn, M.P. Garssen, and K. Geleijns (Erasmus Medical Center, Rotterdam, The Netherlands).

References

1. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
2. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti CL, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
3. Dauvilliers Y, Baumann CR, Maly F, Torchon F, Bassetti CL. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome and other hypersomnia and neurological conditions. J Neurol Neurochir Psychiatry 2003; 74:1667–1673.
4. Ebrahim IO, Sharief KK, De Lacy S, et al. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurochir Psychiatry 2003; 74:127–130.
5. Baumann CR, Dauvilliers Y, Mignot E, Bassetti CL. Normal CSF hypocretin-1 (orexin A) levels in dementia with Lewy bodies associated with excessive daytime sleepiness. Eur Neurol 2004; 52: 73–76.
6. Ripley B, Overeem S, Fujiki N, Nevsimalova S, Uchino M et al. CSF hypocretin/orexin levels in narcolepsy and other neurologic conditions. Neurology 2001; 58:2253–2258.
7. Nishino S, Ripley B, Overeem S, Nevsimalova S, Lammers GJ, Vankova J, Okun M, Rogers W, Brooks S, Mignot E. Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. Ann Neurol. 2001 Sep;50(3):381–388.
8. Kanbayashi T, Yano T, Ishiguro H, Kawanishi K, Chiba S, Aizawa R, Sawaishi Y, Hirota K, Nishino S, Shimizu T. Hypocretin-1 (orexin-A) levels in human lumbar CSF in different age groups: infants to elderly persons. Sleep 2002; 25:337–339.
9. Salomon RM, Ripley B, Kennedy JS, Johnson B, Schmidt D, Zeitzer JM, Nishino S, Mignot E. Diurnal variation of cerebrospinal fluid hypocretin-1 (Orexin-A) levels in control and depressed subjects. Biol Psychiatry 2003; 54:96–104.
10. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, Fujii Y, Nishino S, Shimizu T. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002; 11:91–93.
11. Krahn LE, Pankratz VS, Oliver L, Boeve BF, Silber MH. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25:733–736.
12. Baumann CR, Bassetti CL. Hypocretins (orexins): Clinical impact of the discovery of a neurotransmitter. Sleep Medicine Reviews 2004 (in press).
13. Bassetti CL, Gugger M, Bischof M, Mathis J, Sturzenegger C, Werth E, Radanov B, Ripley B, Nishino S, Mignot E. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.
14. Khatami R, Maret S, Werth E, Retey J, Schmid D, Maly F, Tafti M, Bassetti CL. A monozygotic twin pair concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 363:1199–1200.
15. Kubota H, Kanbayashi T, Tanabe Y, Ito M, Takanashi J, Kohno Y, Shimizu T. Decreased cerebrospinal fluid hypocretin-1 levels near the onset of narcolepsy in 2 prepubertal children. Sleep 2003; 26: 555–557.
16. Overeem N, Van Hilten JJ, Ripley B, Mignot E, Nishino H, Lammers C. Normal hypocretin-1 levels in Parkinson's disease patients with excessive daytime sleepiness. Neurology 2002; 58:498–499.
17. Baumann CR, Ferini-Strambi L, Waldvogel D, Werth E, Bassetti CL. Parkinsonism with excessive daytime sleepiness: A narcolepsy-like disorder? J Neurol 2004 (in press).

18. Drouot X, Moutereau S, Nguyen JP, Lefaucheur JP, Creange A, Remy P, Goldenberg F, D'Ortho MP. Low levels of ventricular CSF orexin/hypocretin in advanced PD. Neurology. 2003; 61:540–543.
19. Overeem S, Dalmau J. Bataller L, Nishino S, Mignot E, Verschuuren J, Lammers GJ. Hypocretin-1 CSF levels in anti-Ma2 associated encephalitis. Neurology 2004; 62:138–140.
20. Nishino S, Kanbayashi T, Fujiki N, Uchino M, Ripley B, Watanabe M, Lammers GJ, Ishiguro H, Shoji S, Nishida Y, Overeem S, Toyoshima I, Yoshida Y, Shimizu T, Taheri S, Mignot E. CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2003; 61:823–825.
21. Baumann CR, Bassetti CL. Normal CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2004; 62:2337.
22. Kubota H, Kanbayashi T, Tanabe Y, Takanashi J, Kohno Y. A case of acute disseminated encephalomyelitis presenting hypersomnia with decreased hypocretin level in cerebrospinal fluid. J Child Neurol 2002; 17:537–539.
23. Gledhill RF, Bartel PR, Yoshida H, Nishino H, Scammell T. Narcolepsy caused by acute disseminated encephalomyelitis. Arch Neurol 2004; 61:758–760.
24. Kato T, Kanbayashi T, Yamamoto K, Nakano T, Shimizu T, Hashimoto T, Ikeda S. Hypersomnia and low CSF hypocretin-1 (orexin-A) concentration in a patient with multiple sclerosis showing bilateral hypothalamic lesions. Intern Med. 2003; 42:743–745.
25. Oka Y, Kanbayashi T, Mezaki T, et al. Low CSF hypocretin-1/orexin-A levels associated with hypersomnia secondary to hypothalamic lesion in a case of multiple sclerosis. J neurol 2004; 251:885–886.
26. Scammell TE, Nishino S, Mignot E, et al. Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke. Neurology 2001; 56:1751–1753.
27. Arii J, Kanbayashi T, Tanabe Y, Ono J, Nishino S, Kohno Y. A hypersomnolent girl with decreased CSF hypocretin level after removal of a hypothalamic tumor. Neurology 2001; 56:1775–1776.
28. Baumann CR, Stocker R, Imhof HG, Mignot E, Trentz O, Bassetti CL. Hypocretin-1 deficiency in acute traumatic brain injury (in revision).

27
CSF Hypocretin-1/Orexin-A in Narcolepsy: Technical Aspects and Clinical Experience in the United States

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

Decreased cerebrospinal fluid (CSF) hypocretin-1 (hcrt-1, orexin A) was first observed in nine patients from the Netherlands with narcolepsy-cataplexy, seven of whom had undetectable hcrt-1 levels (1). Measuring CSF hcrt-1 was later shown to be highly specific and sensitive for narcolepsy-cataplexy (2–8). Rare (10–25%) subjects with narcolepsy without cataplexy and no subjects with idiopathic hypersomnia have been found to have low CSF hcrt-1 (2–8). Hcrt-1 was also measured in the CSF of patients with various neurological and sleep disorders, and levels were shown to be normal or slightly decreased in selected severe neurological disorders (3,7,9). In this chapter, we will briefly review current knowledge pertaining to the use of CSF hcrt-1 measurements in the diagnosis of narcolepsy.

I. CSF Hypocretin-1: Technical Aspects

Initial studies of CSF hcrt-1 measurements were performed after acid extraction and reverse phase Sep-Pak C18 column purification, followed by a radioimmunoassay (RIA) using commercially available polyclonal anti-orexin A antibodies and radiolabeled ^{125}-I-hcrt-1 (Phoenix Pharmaceuticals, Mountain View, California, U.S.A.) (1,9). The peptide extraction procedure increases specificity, as non-peptide impurities interfering with the assay and producing background noise are removed. Problematically, however, material is lost during the extraction and a 1 ml volume (as opposed to only 200 μl for the direct assay described below) is needed to carry out the assay. High performance liquid chromatography (HPLC) elution of CSF extracts followed by RIA assays on successive fractions was performed and elution profiles compared with those of native hcrt-1 (orexin-A). These experiments indicated that most of the detected RIA signal is related to genuine hcrt-1 (with additional derivatives). In extracted assays, the detection limit (IC90-95) falls between 20 pg/ml and 40 pg/ml. We also attempted to measure CSF hypocretin-2 (hcrt-2) but were unsuccessful (9), most likely because of its biological instability, which results in much lower concentrations in the CSF (10).

To simplify the procedure and because high levels could be detected, we next developed a direct RIA assay (9). In this assay, 200 μl of CSF are buffered in duplicate

and directly submitted to the RIA using hcrt-1 polyclonal antibodies (Phoenix Pharmaceuticals, Mountain View, California, U.S.A.). The amount of applied CSF (100 μl × 2) was determined to typically fall within the linear portion of the displacement curve in normal CSF samples. Crude and extracted measurements were carried out using several hundred samples and results were highly correlated (Fig. 1a) (3). Intra-assay differences were found to be below 5% for both the extracted and direct assay. Because of variability in ^{125}-I-hypocretin-1 specific activity and other factors, inter-assay variability was reduced by the addition of several CSF samples of known high and low hcrt-1 values and normalization of each measured value with these standard values (9). The detection limit for the direct assay is similar to that of the extracted assay.

To determine the optimal CSF hcrt-1 value to be used for diagnostic purposes, a large number of patients with and without narcolepsy were tested and Quantitative Receiver Operating Curve analysis was performed (2). Gold standard diagnostic

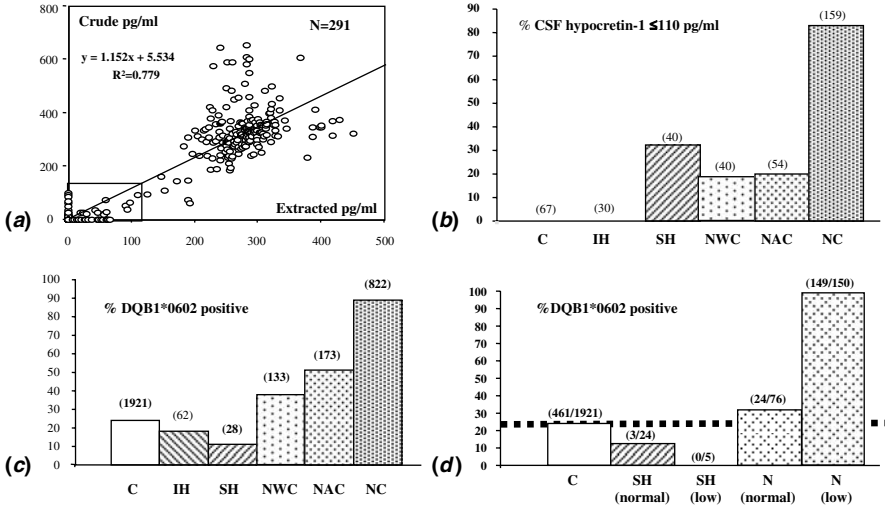


Figure 1 CSF hypocretin-1 measurements: technical aspects and current results in patients with hypersomnia. C: Controls, IH: Idiopathic hypersomnia, SH: Secondary hypersomnia/narcolepsy; NWC: Narcolepsy without cataplexy, NAC: Narcolepsy with atypical/doubtful cataplexy; NC: Narcolepsy with cataplexy, SH (normal): secondary hypersomnia. **(a)** Correlation between CSF hcrt-1 as measured after extraction or using a direct assay. Low CSF values (≤110 pg/ml) represent hypocretin deficient narcoleptic patients. Note high correlation. **(b)** Percentage of patients in various disease categories with low CSF hcrt-1. Note extremely high percentage in narcolepsy with typical cataplexy (80%), dropping to 20–30% in cases with secondary hypersomnia narcolepsy or with atypical or no cataplexy. Unaffected controls and patients with idiopathic hypersomnia were never found to have low CSF hctr-1. **(c)** Percentage of patients with HLA-DQB1*0602 across disease categories. Note the absence of DQB1*0602 association in secondary hypersomnia/narcolepsy, in spite of low CSF hcrt-1 in 30% of the cases **(b)**. **(d)** HLA-DQB1*0602 positivity as a function of hypocretin deficiency. In patients with narcolepsy (independent of cataplexy), almost all subjects with low CSF hcrt-1 are DQB1*0602 positive. Narcolepsy patients with normal or intermediary hcrt-1 levels have a normal or slightly increased DQB1*0602 frequency.

criteria used for narcolepsy were those of the International Classification of Sleep Disorders (ICSD), including both patients with and without cataplexy. Using this criteria, we found that 110 pg/ml, or 30% of mean control values (67 subjects), was the most optimal diagnostic cut-off value. With this cut-off, we found that the test was highly specific for narcolepsy (98%). Sensitivity was 60% overall, extremely high for cases with cataplexy (87%), and decreased significantly in cases without cataplexy (14%) (2).

In this chapter, levels below 110 pg/ml are considered low and above 200 pg/ml are considered normal. All samples reported in this chapter have been tested at Stanford and using the same reference samples so values are comparable (see Workshop report for further discussion). Levels between 110–200 pg/ml are noted as "intermediate," and are inconclusive diagnostically. Further discussion on the technical aspects of the assay and its validation across centers is presented in an associated workshop report.

II. CSF Hypocretin-1: Stability of CSF Measurements, Effects of Drugs, Circadian Time, and Other Manipulations

Surprisingly, we found that the hcrt-1 signal in the human lumbar CSF is remarkably stable and does not exhibit a large rostrocaudal gradient (9), unlike monoamine metabolites. Experiments in which hcrt-1 was measured in successive CSF fractions, after multiple freeze-thaw cycles, or after a 92 hour exposure to room temperature, did not substantially affect measurements (9). We similarly added blood or albumin to the CSF and briefly incubated the samples, but this manipulation also did not change RIA results. The stability of hcrt-1 in vitro is also matched by good stability in vivo, whether in CSF or blood. In CSF experiments where 10 nmoles of hcrt-1 or hcrt-2 are injected in the lateral ventricle, hcrt-1 recovery in the cisterna magna is 100 fold higher than hcrt-2 recovery in the same compartment 2 hours later (10). In blood, the biological half-life of hcrt-1 is 40 minutes (as opposed to 4 minutes for hcrt-2), a relatively long half-life for a peptide, an effect that we believe may be due to the protective nature of the two disulfide bonds present in hcrt-1 (11,12). We, however, still advise freezing of CSF samples within 30 minutes of drawing as a general precaution.

Experiments in rats and squirrel monkeys have shown significant diurnal fluctuations in cisternal CSF levels of hcrt-1, with maximal concentrations at the end of the active period (13). In rodents, cisternal CSF levels closely track in vivo microdialysis release in the medial thalamus and hypothalamus, indicating a primary brain origin. In squirrel monkeys, a species behaviorally close to humans because of its consolidation of sleep and wake, maximal levels in CSF hcrt-1 are observed just prior to sleep onset, with levels decreasing sharply after sleep onset (45% amplitude, peak to trough) (13). In humans, studies in supine individuals, and in a small number of freely moving human volunteers, have shown that CSF hcrt-1 concentrations are only slightly affected by circadian time (6% amplitude, peak to trough), with maximal levels several hours after sleep onset, at around 2 AM (13,14). This profile is likely to reflect the slow equilibration of CSF hcrt-1 levels from the cisterna magna (presumably peaking at bedtime, around 10–11 PM) to the lumbar area (low amplitude peak after midnight). The lack of significant fluctuation offers the advantage of not needing to control for time of the day when testing CSF hcrt-1 to diagnose narcolepsy.

In all species examined to date, sleep deprivation has been shown to increase CSF hcrt-1 (13). This has, unfortunately, not been tested in humans but, if correct, this manipulation would be rather helpful diagnostically as it would have the tendency of further distinguishing patients with insufficient sleep versus narcoleptic subjects.

Other factors that may modulate CSF hcrt-1 include changes in food intake, stress and locomotor activity. As discussed elsewhere, increased activity and food deprivation have been shown to increase cisternal CSF levels in rats but not in squirrel monkeys, suggesting differential effects for these factors in wake-consolidated species (13). Similarly, whereas stress has been shown to increase c-Fos expression in rodent hypocretin neurons, we found no correlations between CSF hcrt-1 and cortisol increases after various manipulations in squirrel monkeys (13). Most strikingly, we recently immobilized our squirrel monkeys for over 12 hours during the daytime and found non-significant changes in CSF hcrt-1, despite the associated stress (112% increase in cortisol) and an 83% decrease in locomotor activity (13,15).

As it is theoretically possible that part of the lumbar CSF hcrt-1 signal may be partially of spinal origin and differentially regulated, additional experiments in humans will be needed to further address the effects of locomotion, stress, and food intake on lumbar concentrations of hcrt-1. It is, for example, possible that large increases in locomotion increase CSF hcrt-1 in humans. Of note however, a comparison of CSF values in normal supine volunteers with intrathecal catheterization and freely moving adults did not support the hypothesis of an effect of immobilization (14). As decreased, not increased, hcrt-1 has diagnostic value, the effect of locomotion is also unlikely to be relevant in a diagnostic context.

The potential effect of psychotropic drugs on CSF hcrt-1 has been scantly studied but evidence to date suggests minor or no effects. CSF hcrt-1 levels in patients with narcolepsy are low or undetectable independent of treatment with antidepressants, amphetamine-stimulants, modafinil or sodium oxybate (1–8). Normal levels are typically observed in patients with idiopathic hypersomnia or narcolepsy without cataplexy, even when treated with the same drugs. In a study of depressed patients, we found that sertraline, a serotonin reuptake inhibitor but not bupropion (primarily a dopamine reuptake inhibitor) decreased CSF hcrt-1 by 14%, independent of the antidepressant treatment response (14). This serotonergic effect is interesting considering the known 5-HT1a inhibitory effect of serotonin on hypocretin neuron electrophysiological activity (16). Similarly, Dalal et al. found that schizophrenic patients treated with haloperidol had 25% lower CSF hcrt-1 levels than untreated patients; patients treated with clozapine or olanzepine had intermediate levels (17). Finally, we found that fibromyalgia patients treated with the alpha-2 agonist tizanidine had increased CSF hcrt-1, an effect that was independent of treatment response (A Larson and E Mignot, unpublished results). As alpha-2 receptors have been shown to inhibit hypocretin activity, it is likely that these effects are neurobiologically indirect (18). A complexity in this area is the potential indirect effect of the associated pathology and/or the effect of the drug on sleep itself, or improved sleep in a given pathology, with an indirect effect on CSF hcrt-1. Nonetheless, all the aforementioned effects are minor, indicating that most of the psychotropic drugs currently used in clinical practice will not interfere with the validity of CSF hcrt-1 for diagnostic purposes.

III. Effects of Other Sleep, Neurologic, and Psychiatric Conditions

In order to assess specificity for low CSF hcrt-1 in narcolepsy, a systematic study of various neuropathologies was initiated (9). Our initial sample survey included both ventricular and lumbar CSF samples. Chronic pathologies, such as Alzheimer's dementia and Parkinson's disease were included. Acute pathologies included various intracerebral tumors, infections, vascular events and traumas. In 226 lumbar samples, the site of CSF draws in narcolepsy, very few samples were within the narcolepsy range (most were >110 pg/ml). Samples included a few cases with Guillain Barré syndrome and one case with a myxoedema coma (3). In the two Guillain Barré cases, the pathology was serious enough that these patients were quadriplegic and under respiratory assistance (9). An extended study of Guillain Barré cases found that in some patients, decreased CSF hcrt-1 occurs prior to severe clinical deterioration with a reversal of the abnormality when the pathology resolved (19), though another study could not confirm these findings (20). Possible interpretations of this finding include hypothalamic involvement in this primarily peripheral neuropathy, an indirect effect of inflammation or autoimmunity on hypocretin cells, or an effect of increased protein in the pathology, with changes in fluid dynamics and signal dilution. Of note, sleep abnormalities, including possible hypnagogic hallucinations and sleep onset rapid eye movement (REM) sleep periods (SOREMPs), have been reported in some patients with Guillain Barré syndrome (I Arnulf, personal communication).

Whereas very low lumbar CSF hcrt-1 levels ($\leqslant 110$ pg/ml) were rarely observed in this survey, a large number of severe and acute pathologies (15% of all samples) had intermediate CSF hcrt-1 levels (110–200 pg/ml) (3,7,9). These included most patients with head trauma, infections and a subset of patients with tumors and other severe pathologies. Whether these results genuinely reflect decreased hypocretin release or changes in peptide stability or changes in CSF flow dynamics is unknown. Post-infectious and posthead trauma sleepiness has been reported and may or may not be related to these changes.

Ventricular samples had generally lower values but it was impossible to interpret this result and these samples were not included in our initial publication (9). Lower levels in ventricular samples may be the result of these samples being obtained from more severe pathologies or/and the effect of draining the CSF from compartments with lower CSF hcrt-1 levels, for example the lateral ventricle, a region less likely to have intense hypocretin projections when compared with the third ventricle. We also anticipate a more significant diurnal fluctuation in levels, as reported in cisternal CSF for squirrel monkeys (45%). Interestingly, we were able to follow the course of diurnal fluctuation every 2 hours in CSF ventricular hcrt-1 in a 60-year old male with a subarachnoid hemorrhage (21). The patient was bedridden, but conscious. Levels were very low initially (60–90 pg/ml) and slowly recovered two weeks after the trauma to reach intermediate (90–130 pg/ml) levels, but the patient finally passed away. Prior to death, however, diurnal fluctuations were more pronounced than those reported in lumbar CSF (peak to trough amplitude 38% versus 6% in lumbar samples) and peaked earlier in the evening (11–12 PM) (21). This result illustrates the complexity of interpreting studies in severely ill patients.

In contrast with findings reported in severe neurological disorders, the study of patients with various sleep and psychiatric disorders (other than hypersomnia) did not reveal any major abnormalities (3,4,6-8,14). Subjects with untreated depression (14) or schizophrenia (22) did not display significant changes. No changes were noted in patients with fatal familial insomnia (23). Normal levels were also found in patients with fibromyalgia syndrome (A Larson, personal communication), sleep apnea and treated restless leg syndrome (3). Slightly elevated levels (+16%) were found in the evening of untreated restless leg subjects (24), an effect that could reflect increased locomotion or sleep deprivation in this disorder (13,15).

In conclusion, our comprehensive survey to date suggests that low CSF hcrt-1 (below 110 pg/ml) is rare outside of narcolepsy. Importantly, however, we also found significantly lower levels (although rarely in the narcolepsy range) in a large range of pathologies associated with a severe neurological impairment and/or loss of consciousness. Changes in CSF hcrt-1 may be most evident in ventricular samples, possibly because of the combined effect of a more severe pathology and CSF circulation effects. The changes observed during acute neurological conditions are likely to be reversible. In my opinion, they are most likely to reflect a temporary inhibition of hypocretin tone by unknown inflammatory substances or environmental changes. These findings argue in favor of conservatively interpreting CSF hypocretin levels when a serious neuropathological association is present.

IV. CSF Hypocretin-1 in Narcolepsy-Cataplexy

A large number of studies performed in Germany, Great Britain, Japan, the Netherlands, Switzerland, Korea, Spain, and the United States have all found that approximately 90% of narcolpetics with cataplexy have low CSF hcrt-1, as defined by undetectable levels or below 110 pg/ml (Fig. 1b). These findings were made in several hundred patients independent of concomitant treatments or association of other sleep disorders. In a smaller number of cases, CSF hcrt-1 was assessed only 1–6 months after a clear disease onset, and in these cases levels were already either very low or undetectable (3,4,25). In almost all cases with low CSF hcrt-1, HLA-DQB1*0602 was found (four exceptions in the world have been reported), illustrating the tight genetic association of hypocretin deficiency with HLA positivity and cataplexy (see ref. 26 and Fig. 1c and d).

In our center, we routinely separate patients with typical, atypical/doubtful and no cataplexy. Cataplexy is considered typical when always of short duration (unless cataplexy is very severe and frequent), and triggered by laughing or joking at least some of the time. Cataplexy is considered doubtful when episodes are very rare (a few times in a life time) or so mild in severity that an actual muscle atonia is difficult to confirm (e.g., when only fleeting sensation is reported or if the patient has to sit down only when laughter is extreme). Cataplexy is classified as atypical when episodes are always prolonged (e.g., 10 minutes) or/and produced by unusual triggers (only anger or stress). Whereas in patients with typical cataplexy CSF hctr-1 was low to undetectable in 90% of cases, subjects with atypical or no cataplexy are only positive for the test 16–30% of the time (Fig. 1b). This finding mirrors earlier observations indicating a very high HLA association (90–95%) in patients with typical and severe cataplexy,

with a progressive decrease to 40% in cases without cataplexy and to 25% in unaffected Caucasian controls (27, see also Fig. 1c).

V. CSF Hypocretin-1 in Narcolepsy Without Cataplexy

Most but not all narcoleptics without cataplexy, but with two SOREMPs on the multiple sleep latency test (MSLT), have normal CSF hcrt-1. Our first case with low CSF hcrt-1 and no cataplexy was a 58 year-old woman with mild sleepiness, sleep paralysis, and vivid dreams who was treated with low doses amphetamine (3). A diagnosis of narcolepsy had been made in the 1960s without a documented MSLT. She strongly denied ever having cataplexy even when laughing or angry and accepted to undergo an MSLT, which showed multiple SOREMPs. This case illustrates the possible existence of genuine narcolepsy cases without cataplexy but with low CSF hcrt-1.

In a recent meta-analysis, we estimated that approximately 25% of 42 patients diagnosed with narcolepsy without cataplexy across multiple specialty clinics with research interest in narcolepsy had low CSF hcrt-1. Forty-five percent of all the narcolepsy without cataplexy cases were HLA-DQB1*0602 positive. All subjects with low CSF hcrt-1 were HLA-DQB1*0602 positive. Forty-five percent of subjects with normal CSF Hcrt-1 were HLA positive, a slightly higher value than the expected 25% population prevalence. These values are slightly higher but similar to those reported in our own database sample (Fig. 1b–d).

Importantly, however, approximately one third of subjects with undetectable CSF hcrt-1 were children and/or subjects with recent onset ($\leq$4 years) likely to develop cataplexy within a few years. Another third, representing 7–10% of the 42 patients, were older adults ($>$50) with a duration of illness longer than 30 years, making it unlikely they will ever develop cataplexy. This result indicates that if patients most likely to develop cataplexy at a later age are removed, only a small portion of adults, possibly 7–10% of currently diagnosed cases, have low CSF hcrt-1. This value may even be lower outside of specialty clinics, where less severe and definite narcolepsy without cataplexy cases, sometimes in association with other pathologies (e.g., residual sleepiness after treatment of sleep apnea), are more likely to be seen. It is also possible that some of these patients have genuine cataplexy but either do not report it, or do not realize it is cataplexy.

VI. Pathophysiological Models of the Narcolepsy Spectrum: Continuum or Heterogeneity

Our current results are consistent with two possible, non-exclusive pathophysiological models (3). In the first model, narcolepsy with and without cataplexy is part of the same disease continuum, with similar pathophysiological effects on hypocretin transmission. Narcolepsy with cataplexy is a generally more severe form of the disease, most commonly associated with an almost complete destruction of the hypocretin system. HLA-DQB1*0602 is a severity factor more likely to predispose to complete hypocretin destruction. Only in rare cases with cataplexy is hypocretin cell loss partial but

sufficient to produce a narcolepsy phenotype, and in these cases, compensation by the remaining cell population typically maintains CSF hcrt-1 at normal levels. Projections with more effects on CSF hcrt-1 but of less functional importance for the phenotype (e.g., to the spinal cord) may be more spared in cases with cataplexy and normal CSF levels. The observation that rare HLA-DQB1*0602 negative cases with cataplexy, who have generally normal CSF hcrt-1, may share an HLA genetic susceptibility with HLA-DQB1*0602 positive cases beyond DQB1*0602—for example, both groups have increased DQB1*0301 frequency—also supports this hypothesis (28).

In narcolepsy without cataplexy, cell loss is less pronounced and cataplexy less likely. The severity of the disease is generally less severe and the cell loss is typically insufficient to result in low CSF hcrt-1. The HLA-DQB1*0602 association is also weaker. Only in rare cases, neuroanatomical destruction is pronounced, resulting in low CSF hcrt-1, but still spares a select hypocretin cell subpopulation projecting to cataplexy triggering pathways. In favor of this model is the report by Thannickal et al. reporting 14% remaining hypocretin cells in a case without cataplexy, versus 4.4–9.4% remaining cells in five other subjects with cataplexy (29). Lesion studies in rats also indicate a 50% decrease in CSF hcrt-1 with a 77% destruction of hypocretin cells, suggesting some degree of compensation (30).

The fact that HLA-DQB1*0602 is slightly increased in frequency in cases without cataplexy but with normal CSF hcrt-1 (Fig. 1d) is also consistent with this hypothesis. This finding however remains to be confirmed in a population based sample as HLA positivity may have been increased in clinical samples due to a bias in inclusion; some clinicians use HLA typing to confirm the diagnosis of narcolepsy without cataplexy. The model also predicts that a large number of narcolepsy without cataplexy cases may exist in the general population but may have a milder phenotype, consistent with our observation of slightly decreased REM latency in normal population subjects with HLA-DQB1*0602 (31). Similar milder forms of the disease or long latent periods have been suggested for other autoimmune diseases such as DQ2 associated celiac diseases (32), B47 associated spondyloarthropathies (33) and DR3/DR4 associated Type I diabetes (34). In Type I diabetes for example, a larger number of individuals, especially relatives of affected probands may be positive for islet cell antibodies without ever developing the disease (33).

In a second model, the cause of cases without low CSF hypocretin does not involve partial hypocretin cell loss (3). Other systems downstream of hypocretin itself, for example, hypocretin receptors, histamine or neuroanatomical systems unconnected with hypocretin neurobiology may be involved. Of note, the two models may not be entirely exclusive. A partial hypocretin cell loss may, for example, not be sufficient in itself to produce symptoms but could when associated with additional defects downstream lead to narcolepsy. A similar model, albeit speculative, has been proposed in some obese type diabetes children where both Types I and II may coexist. In these cases, subjects with partially reduced islet cell numbers may be asymptomatic when lean but develop insulin resistance if obesity develops and the remaining islet population is unable to produce enough insulin to keep up with increased tissue demand.

To distinguish between these models, neuropathological studies of narcolepsy without cataplexy cases are urgently needed. It will also be imperative to study narcolepsy without cataplexy not only in clinical samples but also in the general population as clinical cases may represent a more severe and selected subpopulation. We believe

that the most likely explanation will be a combination of these models (3), including both disease heterogeneity with respect to hypocretin neuropathology and severity gradients for hypocretin cell loss as discussed above. The extent of this overlap remains to be defined.

VII. CSF Hypocretin-1 in Secondary Hypersomnia: Fact or Fiction

Secondary hypersomnia, with or without SOREMPs or cataplexy, has been described as the result of numerous conditions. The importance of the hypothalamus in mediating secondary hypersomnia in encephalitis lethargica was recognized by Von Economo in the 1930s (35,36). Secondary narcolepsy-cataplexy resulting from hypothalamic or diencephalic tumors has also been noted since the early 20th century (36,37). Other potential etiologies for secondary hypersomnia and narcolepsy include genetic disorders, inflammatory and autoimmune disorders, neurodegenerative conditions, head trauma and acute vascular/infectious events (37,38). We have explored CSF hcrt-1 in a number of pathologies associated with sleepiness (Table 1) and found that some, but not all pathologies are associated with decreased CSF hcrt-1 (38). In some cases (e.g., hypothalamic tumors), CSF hcrt-1 may not be as diminished as anticipated, suggesting the potential importance of disrupting specific projections and highlighting the limitation of CSF hcrt-1 as a marker for functionally relevant hypocretin activity (Table 1).

Interestingly, disorders with low CSF hcrt-1 include a number of autoimmune or inflammatory conditions such as Ma-2 paraneoplastic encephalitis and SEART (Table 1). Whether these effects are a true reflection of hypocretin cell destruction or a simple reflection of temporarily suppressed release is unknown. A subset of patients with pleiotropic genetic disorders, for example, myotonic dystrophy and Prader-Willi syndrome, also show very decreased CSF hcrt-1 (Table 1). These results should however be interpreted with caution. As mentioned above, a survey of neurological disorders has shown that low CSF hcrt-1 can be found in the context of severe neurological disorders, most notably inflammatory disorders such as Guillain Barré syndrome and head trauma.

VIII. Diagnostic Utility of CSF Hypocretin-1

The diagnostic specificity of the test is high but utility is strongly limited by the discomfort and possible post-procedure headache associated with carrying out a lumbar puncture (LP) (25). It is also most predictive in cases with cataplexy, when the utility of the test is low. We therefore only recommend using this test in few selected indications (25), preferably using a double barrel thin needle procedure to reduce the occurrence of post-LP headaches.

A primary indication of this test is the need to objectively document a diagnosis when the MSLT cannot be used (associated medications, sleep disorders, inability to follow MSLT directions). This includes already treated patients if the diagnosis is in doubt, for example in the context of drug seeking, malingering or conversion

Table 1 CSF Hypocretin-1 in Secondary Narcolepsy/Hypersomnia Cases

	Hypocretin-1 level (pg/mL)		
	Low ($\leq$110)	Intermediate (110–200)	Normal (>200)
Genetic disorders			
Autosomal dominant cerebellar ataxia, deafness, narcolepsy	1 (0)[a]		
Late-onset central hypovatilation syndrome	1 (0)		
Myotonic dystrophy	1 (0)	2 (0)	3 (0)
Prader-Willi syndrome	1 (0)	2 (0)	1 (1)
Nieman-Pick disease type C		2 (1)	2 (0)
Hypothalamic disorders			
Large pituitary adenoma	1 (nt)[b]		
Hypothalamic lesion post stroke		1 (0)	
Post-resection of a hypothalamic tumor	2 (nt)		
Post-hypothalamic irradiation			1 (0)
Neurocysticercosis cysts hypothalamus/other			1 (0)
Autoimmune disorders			
AntiMa2 Paraneoplastic syndrome	4 (nt)		
Hashimoto's Encephalopathy (SREAT)	1 (nt)		
Infectious			
Central nervous system Whipple's disease	1 (0)	1 (ins)[c]	
HIV encephalopathy with sleepiness			1 (nt)
Others			
Hypersomnia with depression			6 (1)
Hypersomnia with Parkinson's disease			3 (0/2)
Pontine lesion, thalamocortical stroke			2
Post head trauma		1(0)	

[a]Number in parentheses indicates number of HLA-DQB1*0602 positive samples.
[b]Not tested.
[c]This individual with Whipple's disease had severe insomnia rather than hypersomnia. The case is reported to illustrate the difficulties of interpretating decreased CSF hypocretin-1 in neurological pathologies.

disorders. Patients with possible cataplexy (including poor historians or atypical cataplexy) who also have severe sleep apnea or insufficient sleep may also benefit, as the MSLT is not diagnostic. Finally, in young children and in selected cases with associated psychiatric or neurological disorders, the patients may not be able or willing to undergo an MSLT and an LP may be preferable. Narcolepsy-cataplexy patients with a negative MSLT but with high clinical suspicion or patients unusually resistant to anticataplectic or stimulant treatment may also benefit from CSF hcrt-1 measurements.

Other indications are related to the convenience and speed of the assay. Young patients with recent onset without cataplexy may benefit from a rapid and definitive diagnosis if experimental treatments, for example recently discussed immunosuppressive treatments, are considered. Patients unable to pay the full cost of the MSLT, wanting a rapid and definitive diagnosis, or unwilling to stop their medications to complete a diagnostic workup may also prefer a lumbar puncture to the MSLT.

IX. Perspective for a Plasma Assay

A selected number of publications have reported on the measurement of hcrt-1 in the blood (39). Unfortunately, the signal measured in plasma using current polyclonal RIAs is typically at the lower end of the detection limit of the assay. At this level, variation of the signal is high. The needed HPLC and characterization experiments have only rarely been done and it is likely that most, if not all, of the signal is artifactual (39). In this context, most studies have not found differences in plasma hcrt-1-like immunoreactivity levels between narcolepsy and control patients. A Japanese study found lower levels in narcolepsy versus control and good reliability upon repeated measurement, but the difference between narcolepsy and control was small and not discriminative enough for a diagnostic test (39).

Whether circulating hcrt-1 levels exist and can be reliably measured will have to be determined after improving current assay procedures. Interestingly, hcrt-1 is also quite stable in blood (see above). We also found that after perfusion of 2 mg of hcrt-1 into the cisterna magna of a narcoleptic canine, blood hcrt-1 levels were dramatically increased (900 pg/ml), suggesting leakage from the CSF to blood compartment (12). Of note, however, the blood concentration reached was $<10^{-6}$ the estimated CSF concentration after perfusion, suggesting very minimal leakage (12). Whether a potential hcrt-1 signal will be of peripheral (currently disputed) or central origin will also have to be determined and may have important diagnostic consequences if peripheral production is not impaired in human narcolepsy.

X. Conclusion

The use of CSF hcrt-1 measurement has currently a small but growing role in the diagnosis of narcolepsy. To standardize this test worldwide, a process of protocol and reagent sharing, accreditation and training has been set up for centers interested in setting up the technique (40). A major barrier in the widespread use of the assay will remain the need for an LP. We hope that in the future, this procedure will be replaced by plasma based assays, or imaging diagnostic tests.

References

1. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
2. Dalal MA, Schuld A, Haack M, Uhr M, Geisler P, Eisensehr I, et al. Normal plasma levels of orexin A (hypocretin-1) in narcoleptic patients. Neurology 2001; 56:1749–1751.
3. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Archives of Neurology 2002; 59:1553–1562.
4. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, et al. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. Journal of Sleep Research 2002; 11:273.
5. Tsukamoto H, Ishikawa T, Fujii Y, Fukumizu M, Sugai K, Kanbayashi T. Undetectable levels of CSF hypocretin-1 (orexin-A) in two prepubertal boys with narcolepsy. Neuropediatrics 2002; 33:51–52.
6. Krahn LE, Pankratz VS, Oliver L, Boeve BF, Silber MH. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25:733–736.

7. Dauvilliers Y, Baumann CR, Carlander B, Bischof M, Blatter T, Lecendreux M, et al. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. Journal of Neurology Neurosurgery and Psychiatry 2003; 74:1667–1673.
8. Bassetti C, Gugger M, Bischof M, Mathis J, Sturzenegger C, Werth E, et al. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Medicine 2003; 4:7–12.
9. Ripley B, Overeem S, Fujiki N, Nevsimalova S, Uchino M, Yesavage J, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001; 57(12):2253–2258.
10. Yoshida Y, Fujiki N, Maki RA, Schwarz D, Nishino S. Differential kinetics of hypocretins in the cerebrospinal fluid after intracerebroventricular administration in rats. Neuroscience Letters 2003; 346(3):182–186.
11. Kastin AJ, Akerstrom V. Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. Journal of Pharmacology and Experimental Therapeutics 1999; 289(1):219–223.
12. Schatzberg SJ, Cutter-Schatzberg K, Nydam D, Barrett J, Penn R, Flanders J, et al. The effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. Journal of Veterinary Internal Medicine 2004; 18(4):586–588.
13. Zeitzer JM, Mignot E. Role of hypocretin/orexin in the neurobiology of sleep and alertness. In: Narcolepsy and Hypersomnia, Marcel Dekker, in press.
14. Salomon RM, Ripley B, Kennedy JS, Johnson B, Schmidt D, Zeitzer JM, et al. Diurnal variation of cerebrospinal fluid hypocretin-1 (orexin-A) levels in control and depressed subjects. Biological Psychiatry 2003; 54(2):96–104.
15. Zeitzer JM, Buckmaster CL, Lyons DM, Mignot E. Locomotor-dependent and -independent components to hypocretin-1 (orexin A) regulation in sleep-wake consolidating monkeys. Journal of Physiology 2004; 557:1045–1053.
16. Muraki Y, Yamanaka A, Tsujino N, Kilduff TS, Goto K, Sakurai T. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. Journal of Neuroscience 2004; 24(32): 7159–7166.
17. Dalal MA, Schuld A, Pollmacher T. Lower CSF orexin A (hypocretin-1) levels in patients with schizophrenia treated with haloperidol compared to unmedicated subjects. Molecular Psychiatry 2003; 8(10): 836–837.
18. Muhlethaler M. Hypocretin/orexin electrophysiology. In: Narcolepsy and Hypersomnia, Marcel Dekker, in press.
19. Nishino S, Kanbayashi T, Fujiki N, Uchino M, Ripley B, Watanabe M, et al. CSF hypocretin levels in Guillain-Barré syndrome and other inflammatory neuropathies. Neurology 2003; 61(6):823–825.
20. Baumann CR, Bassetti CL. CSF hypocretin levels in Guillain-Barré syndrome and other inflammatory neuropathies. Neurology 2004; 62(12):2337.
21. Brandauer E, Högl B, Lin L, Beer R, Frausher B, Sojer M, et al. Low hypocretin-1 (orexin A) and delayed phase of melatonin in a patient after subarachnoid hemorrhage. Sleep 2003; 26(Suppl):A353.
22. Nishino S, Ripley B, Mignot E, Benson KL, Zarcone VP. CSF hypocretin-1 levels in schizophrenics and controls: relationship to sleep architecture. Psychiatry Research 2002; 110(1):1–7.
23. Martinez-Rodriguez JE, Sanchez-Valle R, Saiz A, Lin L, Iranzo A, Mignot E, Santamaria J. Normal hypocretin-1 levels in the cerebrospinal fluid of patients with fatal familial insomnia. Sleep 2003; 26(8):1068.
24. Allen RP, Mignot E, Ripley B, Nishino S, Earley CJ. Increased CSF hypocretin-1 (orexin-A) in restless legs syndrome. Neurology 2002; 59(4):639–641.
25. Mignot E, Chen W, Black J. On the value of measuring CSF hypocretin-1 in diagnosing narcolepsy. Sleep 2003; 26(6):646–649.
26. Ling L, Mignot E. HLA and narcolepsy: current status. In: Narcolepsy and Hypersomnia, Marcel Dekker, in press.
27. Mignot E, Hayduk R, Black J, Grumet FC, Guilleminault C. HLA DQB1*0602 is associated with cataplexy in 509 narcoleptic patients. Sleep 1997; 11:1012–1020.
28. Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Lin X, et al. Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. American Journal of Human Genetics 2001; 68(3):686–699.
29. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.

30. Gerashchenko D, Murillo-Rodriguez E, Lin L, Xu M, Hallett L, Nishino S, et al. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Experimental Neurology 2003; 184:1010–1016.
31. Mignot E, Young T, Lin L, Finn L, Palta M. Reduction of REM sleep latency associated with HLA-DQB1*0602 in normal adults. Lancet 1998; 351(9104):727.
32. D'Archivio M, Silano M, Fagnani C, Scazzocchio B, Nistico L, Giovannini C, et al. Clinical evolution of celiac disease in Italy 1982–2002. Journal of Clinical Gastroenterology 2004; 38(10):877–879.
33. Braun J, Bollow M, Remlinger G, Eggens U, Rudwaleit M, Distler A, Sieper J. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. Arthritis and Rheumatology 1998; 41:58–67.
34. Knip M. Natural course of preclinical type 1 diabetes. Hormone Research 2002; 57(Suppl 1):6–11.
35. Von Economo CV. Sleep as a problem of localization. Journal of Nervous and Mental Disease 1930; 71(3):249–259.
36. Daniels LE. Narcolepsy. Medicine 1934; 13(1):1–122.
37. Aldrich MS, Naylor MW. Narcolepsy associated with lesions of the diencephalon. Neurology 1989; 39(11):1505–1508.
38. Mignot E. Pathophysiology of narcolepsy. In: Principles and Practice of Sleep Medicine (Kryger M, Roth T, Dement WC, eds), New York: William Sanders, 2005; in press.
39. Nishino S, Mignot E. Article reviewed: Plasma orexin-A is lower in patients with narcolepsy. Sleep Medicine 2002; 3(4):377–378.
40. Mignot E, Bassetti C, Billiard M, Dauvilliers Y, Kanbayashi T, Lammers GJ, et al. Consensus based guidelines for the measurements of CSF hypocretin-1 in the diagnosis of narcolepsy. In: Narcolepsy and Hypersomnia, Marcel Dekker, in press.

28
Hypocretin Pathology in Human Narcolepsy

THOMAS C. THANNICKAL and JEROME M. SIEGEL
Neurobiology Research, VA GLAHS Sepulveda, UCLA Department of Psychiatry, David Geffen School of Medicine, Los Angeles, California, U.S.A.

I. Introduction

Most cases of narcolepsy are caused by abnormalities of the hypocretin (Hcrt) system. Human narcolepsy is most likely due to postnatal degeneration of Hcrt cells through an inflammatory process. The most reasonable hypothesis explaining Hcrt cell loss is that it is immune system mediated. Intravenous administration of Hcrt can reverse the symptoms of narcolepsy. Variability in symptom expression among human narcoleptics, and even among animal mutant narcoleptics, remains to be fully explained and most likely involves interactions between Hcrt neurons and brain aminergic and amino acid systems.

In 2000, two papers identified the loss of Hcrt cells as the cause of human narcolepsy (1,2). Both papers also concluded that melanin concentrating hormone cells were present in approximately normal numbers in the regions in which Hcrt cells are typically found. This suggested that the loss of Hcrt cells was relatively specific; that adjacent melanin-concentrating hormone cells were spared by whatever process was responsible for the loss of Hcrt cells. The specificity of cell loss is consistent with the hypothesis that narcolepsy is an autoimmune disease. This hypothesis was first suggested by Honda, who discovered the HLA linkage of narcolepsy (3). Since HLA linked diseases have been found to have an autoimmune etiology, Honda hypothesized that narcolepsy was caused by immune system damage to the CNS. This hypothesis was given greater specificity by the finding of a discrete loss of Hcrt cells.

Although the general conclusions of the Thannickal et al. (2) and Peyron et al. (1) papers were similar, the differences in the details of the findings of these papers have important implications for understanding the etiology of the disease. The Peyron et al. paper reported that there was no visible gliosis in the narcoleptic brains compared to controls. However, Thannickal et al. reported greatly elevated gliosis in hypothalamic regions, with numbers of astrocytes in the hypothalamus of narcoleptic humans that were double or triple those of control brains. Thannickal et al. also reported that levels of gliosis in thalamic regions did not differ between narcoleptic and normal brains.

The presence of gliosis indicates that Hcrt cells were lost due to an inflammatory process, rather than a developmental or apoptotic process. This would be consistent

with the idea that human idiopathic narcolepsy is caused by an autoimmune attack. We compared the intensity of gliosis in narcoleptic brains in the hypothalamic regions in which Hcrt cells had been lost and in the Hcrt projection regions (4). We found that although gliosis was intense in Hcrt cell loss regions, it was equally or more intense in certain projection regions that were devoid of Hcrt cell somas such as paraventricular, periventricular, arcuate and tuberomammillary nucleus. When we investigated the pattern of gliosis we found that it was most intense in regions which contained Hcrt receptor 2. It was also particularly intense in regions with high concentrations of Hcrt axons in the normal brain. The number of axons counted with the unbiased counting frames yields an unbiased estimate of the total number of axons. By identifying the axon hillock or by excluding tapering processes each axon could be distinguished from dendritic branches. Dendrites have many processes and their surface is irregular and covered in dendritic spines whereas axons have varicosities. The numerical density of Hcrt axons were calculated as number of axons per unit area (mm^2) (4). Our statistical analysis indicated that gliosis was linked to the density of Hcrt receptor 2 and to axonal density, and that these two correlations were independent. A unifying hypothesis that incorporates these findings states that the type 2 Hcrt receptor or an antigen that is anatomically linked to it is the target for an autoimmune attack that ultimately kills Hcrt cells and that this immune reaction is intensified in regions of high axonal density.

Another difference between the Peyron et al. and the Thannickal et al. papers is that the latter reported the presence of surviving cells in all narcoleptics, although the overall number of cells was reduced on average by 90%. In contrast, the Peyron et al. paper reported that all Hcrt cells were lost. The latter observation coincided with results from assays of Hcrt levels in the cerebrospinal fluid of narcoleptics (5). However, both observations have been limited by the sensitivity of the assays used. In situ autoradiography, used for the detection of Hcrt cells is less sensitive than immunohistochemistry. Immunohistochemistry labels the antigen in an all or none process if a specific antigen is used and procedures are properly followed. In our experience this procedure produces highly reproducible number of neurons when sampled with unbiased stereology in repeated measures of the same or several normal brains. In contrast, in in situ labeling, the labeling is graded and thresholds have to be established ad hoc and be compared with control tissue. Thus although relative numbers can be determined, absolute number and issue of presence or absence of particular cell types cannot be made with great confidence. Likewise, the report that Hcrt CSF levels are below detectable limits may be a function of the sensitivity of the assay. We predict that more sensitive assays will show greatly reduced, but still detectable, Hcrt levels in most human narcoleptics. Residual Hcrt function can explain the much less severe cataplexy in humans than is observed in knockout animals, which completely lack Hcrt. The variability in the location and projections of Hcrt cells lost, may also affect the relative intensity of symptoms expressed in human narcolepsy, although this has yet to be documented.

Hcrt levels can affect the intensity of narcoleptic symptoms. We administered Hcrt-1 intravenously to canine narcoleptics and found a significant reduction in symptoms, with increased consolidation of waking activity and reduced cataplexy, as well as a reduction in REM sleep comparable to that seen with intracerebroventricular Hcrt (6). In recent work these same effects have been seen in the idiopathic

narcoleptic dogs with presumed Hcrt cell loss, and also in genetically narcoleptic (Hcrtr-2 mutant) dogs (7,8). In the latter, a significant suppression of REM sleep was reported after IV administration of Hcrt. Although the latter studies used much higher doses of Hcrt, both studies indicate that systemic Hcrt administration is effective, and that administration of effective doses produces no apparent side effects.

Because increasing Hcrt levels by IV administration reduced narcoleptic symptomatology, and because exercise increased Hcrt levels (9), we wondered whether exercise would reduce cataplexy levels. We found that this was indeed the case. Exercise decreased cataplexy with a time course mirroring that of exercise induced shifts in Hcrt level (10). We also find that physostigmine and prazosin given in doses that increase cataplexy greatly reduce Hcrt level. Conversely, labetalol, phenylephrine and methamphetamine, all of which reduce narcoleptic symptomatology, increase Hcrt levels. However, atropine, which reduces cataplexy, does not affect Hcrt levels, presumably acting "downstream" from Hcrt (11).

Although these data clearly indicate a tight linkage between Hcrt levels and narcoleptic symptomatology, the level of Hcrt and even the genetic integrity of the Hcrt system is not the sole determinant of the nature and severity of symptomatology. There is a marked developmental variation in symptom intensity in genetically narcoleptic Hcrtr-2 mutant dogs. Dogs are not symptomatic at birth and gradually become symptomatic starting at 1–2 months of age. Symptoms peak in intensity and gradually diminish with age. Some older dogs show no cataplexy by 2–3 years of age, although symptoms can be reinstituted by administration of physostigmine or prazosin. These drugs produce no cataplexy when administered alone or in combination to normal dogs, even at very high doses. A recent study of the developmental changes in Hcrt levels show that adult Hcrt levels are present at birth and although these levels change over the course of development, these changes cannot by themselves explain the changes in symptoms with age (12).

In an additional study, we found that treatment of narcoleptic dogs with methylprednisolone and methotrexate starting at birth dramatically delayed and greatly reduced symptoms of narcolepsy compared to littermates (13). This reduction was not a direct drug effect of these immunosuppressant drugs. Transient treatment with these drugs for up to two weeks had no significant effect on cataplexy and other measures of narcoleptic symptomatology. However, chronic treatment from birth to the age of 4–6 months produced an apparently permanent reduction in symptoms in otherwise normally active and healthy animals. This treatment effect may have been mediated by immune system interactions with neural development or by direct long-term effects of the administered drugs on the brain degenerative processes in the developing narcoleptic brain (14). It illustrates that the genetically determined trait of narcolepsy can be strongly modulated by drug administration, produced changes in the brain.

These demonstrations of variability in symptom expression in narcoleptic animals are of great relevance to understanding and treating human narcoleptics. Humans with apparently identical Hcrt depletion can have severe cataplexy or no cataplexy at all. One explanation of this variability is that, although overall Hcrt depletion is similar, patients may have a heterogeneous pattern of cell loss, and each pattern of cells loss may be associated with a distinctive set of symptoms. Another explanation

is that the primary determinant of the symptomatology resulting from Hcrt depletion depends on indirect effects of Hcrt loss on brain functioning which are distinctive in each individual. Cases of narcolepsy with normal Hcrt level have also been identified (5). These cases suggest that disruption of systems affected by Hcrt can cause symptoms of narcolepsy even without any Hcrt cell loss. Another explanation of this phenomenon is that there may be other causes of narcolepsy independent of Hcrt pathology. Hypocretin neurons have been shown to powerfully drive aminergic neurons (15–17) and has been shown to produce glutamate release (18,19). Therefore, the loss of both direct and indirect effects of hypocretin are likely to be responsible for the symptoms of narcolepsy.

Acknowledgments

Supported by, PHS grants NS14610, MH64109, HL060296 and the Medical Research Service of the Dept. of Veterans Affairs. Additional references can be found at: http://www.npi.ucla.edu/sleepresearch.

References

1. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
2. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
3. Honda Y, Doi Y, Juji T, Satake M. Narcolepsy and HLA: Positive DR2 as a prerequisite for the development of narcolepsy. Folia Psychiatr Neurol Jpn 1984; 38:360.
4. Thannickal TC, Siegel JM, Nienhuis R, Moore RY. Pattern of hypocretin (orexin) soma and axon loss, and gliosis, in human narcolepsy. Brain Pathol 2003; 13:340–351.
5. Ripley B, Overeem S, Fujiki N, Nevsimalova S, Uchino M, Yesavage J, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001; 57:2253–2258.
6. John J, Wu MF, Siegel JM. Hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep 2000; 23:A12.
7. Siegel JM. Hypocretin administration as a treatment for human narcolepsy (Editorial). Sleep 2003; 26:932–933.
8. Fujiki N, Yoshida Y, Ripley B, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin-ligand-deficient narcoleptic dog. Sleep 2003; 26:953–959.
9. Wu MF, John J, Maidment N, Lam HA, Siegel JM. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. Am J Physiol Regul Integr Comp Physiol 2002; 283:R1079–R1086.
10. John J, Wu MF, Maidment N, Lam HA, Boehmer LN, Patton M, et al. Increased activity reduces cataplexy in canine narcolepsy: Possible role of elevated hypocretin level. Sleep 2004; 27:049.
11. Wu MF, Nienhuis R, John J, Maidment N, Lam HA, Boehmer LN, et al. Effects of drugs affecting blood pressure and heart rate on the release of hypocretin in normal dogs. Sleep 2004; 27:077.
12. John J, Wu MF, Maidment NT, Lam HA, Boehmer LN, Patton M, et al. Developmental changes in CSF hypocretin-1 (orexin-A) level in normal and genetically narcoleptic Doberman pinschers. J Physiol 2004; 560:587–592.
13. Boehmer LN, Wu MF, John J, Siegel JM. Treatment with immunosuppressive and anti-inflammatory agents delays onset of canine genetic narcolepsy and reduces symptom severity. Exp Neurol 2004; 188:292–299.

14. Siegel JM, Nienhuis R, Gulyani S, Ouyang S, Wu MF, Mignot E, et al. Neuronal degeneration in canine narcolepsy. J Neurosci 1999; 19:248–257.
15. Horvath TL, Peyron C, Diano S, Ivanov A, Aston-Jones G, Kilduff TS, et al. Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. J Comp Neurol 1999; 415:145–159.
16. Kiyashchenko LI, Mileykovskiy BY, Lai YY, Siegel JM. Increased and decreased muscle tone with orexin (hypocretin) microinjections in the locus coeruleus and pontine inhibitory area. J Neurophysiol 2001; 85:2008–2016.
17. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. Annual Rev of Psychol 2004; 55:125–148.
18. Peever JH, Lai YY, Siegel JM. Excitatory effects of hypocretin-1 (orexin-A) in the trigeminal motor nucleus are reversed by NMDA antagonism. J Neurophysiol 2003; 89:2591–2600.
19. van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci 1998; 18:7962–7971.

29
Symptomatic Narcolepsy with Cataplexy and Without Cataplexy or Hypersomnia, with and Without Hypocretin (Orexin) Deficiency

T. KANBAYASHI, M. ABE, YASUO HISHIKAWA, and TETSUO SHIMIZU
Department of Neuropsychiatry, Akita University School of Medicine, Akita, Japan

SEIJI NISHINO
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

Narcolepsy is a chronic sleep disorder characterized by excessive daytime sleepiness (EDS), cataplexy, hypnagogic hallucinations (HH), and sleep paralysis (SP) (i.e., narcolepsy tetrad) (1,2). A major break through in narcolepsy research was recently made through the identification of hypocretin deficiency in narcolepsy-cataplexy (2–9). Hypocretins are hypothalamic neuropeptides involved in various fundamental hypothalamic functions including, sleep-wake control, energy homeostasis, autonomic and neuroendocrine functions (10–12). Hypocretin containing neurons are located exclusively in the lateral hypothalamic area (LHA). Since hypocretin deficiency in narcolepsy is also tightly associated with human leukocyto antigen (HLA) DR2/DQ6 (DQB1*0602) positivity, an acquired cell loss of hypocretin containing neurons with autoimmune process are suggested in "so-called" idiopathic cases of narcolepsy (2,6). "Idiopathic narcolepsy" has been used for the cases with narcolepsy unassociated with apparent radiographical or clinical evidence of brain pathology apart from sleep-related abnormalities. Hypocretin deficiency in the brain can be determined clinically via cerebrospinal fluid (CSF) hypocretin-1 measures with CSF hypocretin-1 levels in healthy subjects above 200 pg/ml regardless of gender, age (from neonatal to 70s), and time of the CSF collections (1,4,6). Due to the specificity and sensitivity of low CSF hypocretin-1 levels (less than 110 pg/mL or 30% of the mean normal levels) narcolepsy-cataplexy is high among various sleep disorders (2,13,14), CSF hypocretin measures will be included in the diagnostic criteria for narcolepsy-cataplexy in the 2nd revision of international classification of sleep disorders (ICSD).

Impaired hypocretin systems may also be observed in some neurological disorders affecting the LHA (where hypocretin cell bodies locate) and hypocretin projection pathways. Indeed, an earlier study by Ripley et al. (13) had measured CSF hypocretin levels in 235 neurological patients and shown that a subset of subjects with acute or sub-acute neurological disorders [i.e., intracranial tumors, cerebrovascular events, craniocerebral trauma, central nervous system (CNS) infections, and Guillain-Barré Syndrome (GBS)]

had decreased CSF hypocretin-1 level, although CSF hypocretin-1 levels in the majority of patients with chronic neurological conditions, such as Alzheimer's disease and Parkinson disease, are not significantly reduced. Arii et al. (15) also recently studied CSF hypocretin-1 levels in 132 pediatric neurological conditions. The results are consistent with Ripley's study (13), and only a limited number of neurological conditions beside narcolepsy showed reduced levels. These include intracranial tumors (15), craniocerebral trauma and autoimmune and post-infectious disease [GBS and acute disseminated encephalomyelitis (ADEM)] and in some inherited disorders, such as Niemann-Pick disease, type C (NPC) and Prader-Willi syndrome (PWS) (15).

The findings by Ripley et al. (13) and Arii et al. (15) are particularly interesting since these neurological conditions are often associated with acutely disturbed consciousness, lethargy, sleepiness, and/or residual sleep disturbances. In rare cases, symptoms of narcolepsy can be seen during the course of a neurological disease process (i.e., symptomatic narcolepsy). Interestingly, involvements of the hypothalamic structures in symptomatic narcoleptic cases are emphasized repeatedly from several decades ago (16,17), and impaired hypocretin system may also be involved in some symptomatic narcolepsy cases. Association with EDS/cataplexy in some inherited neurological diseases (such as NPC, PWS, or myotonic dystrophy) is also known (18–20). An impaired hypocretin system may thus also be involved in these sleep-related symptoms in conjunction with these neurological conditions.

In this chapter, we first overview cases of symptomatic narcolepsy reported in literature. Since EDS without other narcolepsy symptoms can also occur with a variety of neurological disorders and are not usually an indication of narcolepsy, we will also extend our discussion on the roles of hypocretin system in EDS disorders associated with various neurological conditions.

Since data of CSF hypoocretin-1 measures are available for some recent symptomatic narcolepsy and/or EDS cases, we will focus on these cases and discuss the roles of hypocretin status in these disorders (Table 1). For this purpose, we categorized the cases as follows: (I) symptomatic narcolepsy-cataplexy associated with focal/generalized CNS invasion, such as cerebral tumors, vascular diseases (Sections IIA), and neurodegenerative disorders (Sections IIB), (II) hypersomnia associated with (IIa) focal/generalized CNS invasion, such as cerebral tumors, brain infections, vascular diseases, neurodegenerative disorders (AD and PD) and head trauma (Section IIIA), and (IIb) with CNS diseases mediated with neuroimmune mechanisms, such as inflammatory and demyelinating diseases (Section IIIB). Non-narcoleptic hypersomnia categories include less defined EDS cases, and likely consists of heterogeneous conditions. This is partially due to the fact that applying standardized polygraphic assessments [all night polygraphic recordings followed by multiple sleep latency test (MSLT)] was often difficult in these neurological conditions. However, since prevalence of these hypersomnia cases appeared to be much higher than that of symptomatic narcolepsy, we believe that the discussion on the roles of the hypocretin system in less well-defined EDS cases also have clinical implications.

A. Definition of Symptomatic Narcolepsy and Its Overview

Symptoms of narcolepsy can be sometime seen during the course of a neurological disease process. In such instances, the term "symptomatic narcolepsy" is used, implying that narcolepsy is a symptom of the underlying process rather than idiopathic. In

Table 1 Symptomatic Narcolepsy or EDS with Hypocretin Measurements

	Lesion	Location	Age	Gender	Narcoleptic Symptoms EDS	Sleep latency	SOREMP	CA	HLA DR2/DQB1*0602(DQw1)	Hypocretin		Note	References	Author
Tumors ($n = 10$)														
E	Astrocytoma resection	Hypothalamus	16	f	+	1.7 m/MSLT	−	−	−	Low	104'pg/mL		57	Arii (2001)
N	Astrocytoma resection	Suprasellar	11	m	+	?	+	−	−	Low	<40 pg/mL		54,101	Marcus (2002)
E	Craniopharyngioma	Hypothalamus	mean 15	m = 2, f = 3	+	mean:10.3 m/MSLT	?	−	?	Control level	mean = 133 pg/mL		55	Snow (2002)
E	Craniopharyngioma	Hypothalamus	mean 15	m = 2, f = 3	+	mean:10.3 m/MSLT	?	−	?	Control level	mean = 133 pg/mL		55	Snow (2002)
E	Craniopharyngioma	Hypothalamus	mean 15	m = 2, f = 3	+	mean:10.3 m/MSLT	?	−	?	Control level	mean = 133 pg/mL		55	Snow (2002)
E	Germinoma	Hypothalamus	mean 15	m = 2, f = 3	+	mean:10.3 m/MSLT	?	−	?	Control level	mean = 133 pg/mL		55	Snow (2002)
E	Arachnoid cyst	Thalamus	mean 15	m = 2, f = 3	+	mean:10.3 m/MSLT	?	−	?	Control level	mean = 133 pg/mL		55	Snow (2002)
N	Choroid plexus carcinoma resection	Pineal gland, thalamus	28	f	+	7.5 m/MSLT	+	−	−	Normal	518 pg/mL		58	Krahn (2002)
NC	Adenoma	Pituitary, hypothalamus	60	m	+	6.4 m/MSLT	+	+	−	Normal	275 pg/mL	Acromegaly +	59	Dempsey (2003)
NC	Tumor	Hypothalamus	65	f	+	5m/1EEG	+	+	?	Low	61 pg/mL		60	Nokura (2004)
Head trauma ($n = 3$)														
E	Head trauma	Non-specific	23	m	+	4.5 m/MSLT	−	−	−	Intermediate	176 pg/mL		67	Dauvilliers (2003)
E	Head trauma	Non-specific	21	m	+	3 m/MSLT	−	−	+	Normal	503 pg/mL		67	Dauvilliers (2003)
E	Head trauma	Base of skull	15	m	+	2m/1EEG	−	−	?	Intermediate	151 pg/mL	GCS12	68	Arii (2004)
Vascular disorders ($n = 5$)														
NC	Infarction	Hypothalamus	23	m	+	0.5 m/MSLT	+	+	−	Intermediate	167 pg/mL		86	Scammell (2001)
E	Infarction	Thalamus	34	m	+	9 m/MSLT	−	−	?	Normal	265 pg/mL		8	Bassetti (2003)
E	Infarction	Ponto-medullary	40	m	+	1 m/MSLT	−	−	?	Normal	316 pg/mL		8	Bassetti (2003)
N	Infarction	Thalamus	45	m	+	5 m/MSLT	+	−	?	Normal	312 pg/mL		60	Nokura (2004)
E	Infarction	Thalamus	15	m	+	?	−	−	?	Normal	274 pg/mL		103	Tohyama (2004)
Encephalopathies ($n = 3$)														
NC	Rasmussen's syndrome	Left frontotemporal and insular	40	m	+	1.6 m/MSLT	+	+	+	Low	<40 pg/mL		108	Lagrange (2003)
E	Wernicke encephalitis	Hypothalamus	5	f	+	?	−	−	?	Low	<40 pg/mL		106	Kashiwagi (2004)
E	Limbic encephalitis	Limbic, hypothalamus	65	m	+	?	−	−	?	Low	87 pg/mL		107	Yamato (2004)

(*Continued*)

Table 1 Symptomatic Narcolepsy or EDS with Hypocretin Measurements (*Continued*)

	Lesion	Location	Age	Gender	Narcoleptic Symptoms EDS	Sleep latency	SOREMP	CA	HLA DR2/DQB1*0602(DQw1)	Hypocretin		Note	References	Author
Degeneration ($n = 33$)														
E	Parkinson's disease	Non-specific	69	m	+	6.1 m/MSLT	−	−	−	Normal	253 pg/mL		109	Overeem (2002)
N	Parkinson's disease	Non-specific	64	m	+	4.9 m/MSLT	+	−	−	Normal	307 pg/mL		109	Overeem (2002)
E	Parkinson's disease	Non-specific	52	m	+	4.4 m/MSLT	−	−	−	Normal	319 pg/mL		109	Overeem (2002)
E	Parkinson's disease ($n = 16$)	Non-specific	?	?	+	?	?	−	?	Low	<50–97 pg/mL		110	Drouot (2003)
E/REF	Parkinson's disease ($n = 3$)	Non-specific	?	?	+/−	?	?	−	?	Intermediate	138–169 pg/mL		110	Drouot (2003)
E	Dementia with Lewy bodies ($n = 10$)	Cortical atrophy	69–82	m:7,f:3	+	?	−	−	?	Normal	382–667 pg/mL		111	Baumanna (2004)
E	Progressive supranuclear palsy	Enlargement of the 3rd V	74	f	+	2.0 m/MSLT	−	−	+	Low	<40 pg/mL		112	Hattori (2003)
Heredogenerative disorders ($n = 1$)														
NC	ADCA-DN	Enlargement of the 3rd V, brainstem atrophy	51	m	+	?	?	+	−	Low	96 pg/mL		84	Melberg (2001)
Immnune-mediated Demyelinating disorders ($n = 7$)														
N	MS	Hypothalamus	22	f	+	2.8 m/MSLT	+	−	−	Low	<40 pg/mL		74,75	Iseki (2002), Oka (2004)
E	MS	Hypothalamus	45	f	+	?	−	−	?	Low	<40 pg/mL		116	Kato (2003)
E	MS	Hypothalamus	43	f	+	?	−	−	?	Intermediate	191 pg/mL		121	Nozaki (2004)
E	ADEM	Hypothalamus	12	f	+	4.5 m/MSLT	−	−	−	Low	102 pg/mL		117	Kubota (2002)
N	ADEM	Hypothalamus, coronaradiata, aqueduct, raphe	38	f	+	4.4 m/MSLT	+	−	+	Low	87 pg/mL		118	Gledhill (2004)
E	ADEM	Hypothalamus	7	f	+	?	−	−	−	Intermediate	146 pg/mL		119	Yoshikawa (2004)
E	ADEM	Hypothalamus	0.9	f	+	?	−	−	?	Low	<40 pg/mL		120	Yano (2004)
Immune-mediated polyneuropathy ($n = 2$)														
E	Guillain-Barre syndrom	Non-specific	28	m	+	0.7m/TNST	−	−	?	Low	<40 pg/mL		126	Nishino (2003)

E	Guillain-Barre syndrom	Non-specific	19	m	+	0.8m/TNST	−	−	?	Intermediate	151 pg/mL		126	Nishino (2003)
Paraneoplastic autoimmune syndromes ($n = 6$)														
E	Anti-Ma associated encephalitis	Lesions in both mesiotemporal regions, ventricular enlargement, temporal lobe atrophy	45	m	+	?	?	−	?	Low	<100 pg/mL	Non-seminomatous germ cell tumor of the testis	128	Overeem (2004)
E	Anti-Ma associated encephalitis	Left temporal enhancing abnormalities	22	m	+	?	?	−	?	Low	<100 pg/mL	Mixed germ-cell tumor of the testis	128	Overeem (2004)
REF	Anti-Ma associated encephalitis	Brainstem, periventricular region, basal ganglia	82	f	−	?	?	−	?	Normal	237 pg/mL	Poorly differentiated lung cancer	128	Overeem (2004)
E	Anti-Ma associated encephalitis	Thalamus, superior collicular, medial temporal lesions	67	f	+	?	?	−	?	Low	<100 pg/mL	Adenocarcinoma of the lung	128	Overeem (2004)
E	Anti-Ma associated encephalitis	Hippocampus, midbrain	38	m	+	?	?	−	?	Low	<100 pg/mL	Germ-cell tumor of the testis (seminoma)	128	Overeem (2004)
REF	Anti-Ma associated encephalitis	Non-specific	53	f	−	?	?	−	?	Normal	218 pg/mL	Adenocarcinoma of the ovary	128	Overeem (2004)
Genetic/Congenital disorders ($n = 14$)														
E	PWS	Non-specific	16	m	+	3 m/MSLT	−	−	−	Low	109 pg/mL	BMI 48.1, AHI 5.6	2	Mignot (2002)
E	PWS	Non-specific	10	m	+	6 m/MSLT	−	−	?	Intermediate	130 pg/mL	BMI29.8, AHI 3.1	92	Nevsimalova/2005
REF	PWS	Non-specific	23	m	−	?	−	−	?	Intermediate	191 pg/mL	BMI49, AHI 46.8	92	Nevsimalova/2005
REF	PWS	Non-specific	6	m	−	?	−	−	?	Normal	226 pg/mL	BMI 25.8, AHI0	92	Nevsimalova/2005
REF	PWS	Non-specific	0.5m	m	?	?	−	−	?	Intermediate	192 pg/mL		15	Arii (2004)
C	NPD	Non-specific	5	m	−	16.5m/TNST	−	+	−	Intermediate	142 pg/mL		95	Kanbayashi (2003)
NC	NPC	Non-specific	14	f	+	5.1 m/MSLT	+	+	+	Intermediate	157 pg/mL		93	Vankova (2003)

(*Continued*)

Table 1 Symptomatic Narcolepsy or EDS with Hypocretin Measurements (*Continued*)

	Lesion	Location	Age	Gender	Narcoleptic Symptoms EDS	Sleep latency	SOREMP	CA	HLA DR2/DQB1*0602(DQw1)	Hypocretin		Note	References	Author
E	NPC	Non-specific	25	f	+	3.5 m/MSLT	−	−	−	Normal	226 pg/mL		93	Vankova (2003)
E	NPC	Non-specific	24	m	+	3.2 m/MSLT	−	−	−	Normal	245 pg/mL		93	Vankova (2003)
E	NPC	Non-specific	31	m	+	10.7 m/MSLT	−	−	−	Intermediate	190 pg/mL		93	Vankova (2003)
N	MYD	Non-specific	46	?	+	4.7 m/MSLT	+	−	−	Low	91 pg/mL		20	Martinez (2003)
E	MYD	Non-specific	47	?	+	7 m/MSLT	−	−	−	Normal	200 pg/mL		20	Martinez (2003)
E	MYD	Non-specific	19	?	+	8 m/MSLT	−	−	−	Intermediate	187 pg/mL		20	Martinez (2003)
N	MYD	Non-specific	50	?	+	1.8 m/MSLT	+	−	−	Normal	206 pg/mL		20	Martinez (2003)
E	MYD	Non-specific	25	?	+	5.7 m/MSLT	−	−	−	Normal	235 pg/mL		20	Martinez (2003)
E	MYD	Non-specific	68	?	+	5.7 m/MSLT	−	−	−	Intermediate	167 pg/mL		20	Martinez (2003)
N	MYD	Non-specific	23	m	+	6.4 m/MSLT	+	−	−	Normal	401 pg/mL		67	Dauvilliers (2003)
Others ($n = 2$)														
E	Hashimoto's encephalopathy	?	65	m	+	?	−	−	?	Low	<40 pg/mL	Full recover by steroid	131	Castillo (2004)
O	Whipple's disease	?	53	m	?	?	−	−	?	Intermediate	113 pg/mL		132	Voderholzer (2002)

Abbreviations: NC; Symptomatic Narcolepsy-Cataplexy; N, Symptomatic Narcolepsy; E, Symptomatic EDS; C, Symptomatic Cataplexy; O, Other; REF, Reference cases (no EDS); EDS, Excessive daytime sleepiness; CA, Cataplexy; V, Ventricle; MSLT, multiple sleep latency test; TNST, two-nap sleep test; EEG, electroencephalogram; MS, multiple sclerosis; ADEM, acute disseminated encephalomyelitis; PWS, Prader-Willi Syndrome; MYD, myotonic dystrophy; +, present; −, absent; ?, Not assessed; ADCA-DN, autosomal dominant cerebellar ataxia, deafness and narcolepsy.

this case, the signs and symptoms of narcolepsy should be temporally associated with underlying neurological process. Many authors use symptomatic narcolepsy and secondary narcolepsy indiscriminately, even though they have apparently different meanings. We suggest the use of symptomatic narcolepsy/EDS, since "secondary EDS" has also been used for EDS associated with sleep apnea and restless leg syndrome.

Although several important original studies and extensive reviews (21–29) for symptomatic narcolepsy are available, many older cases have no objective measures for sleepiness, and the diagnosis of these cases mostly based on the clinical criteria (30–38). Furthermore, some of these reports did not provide the symptomatology and course of the assumed causal disease.

The current diagnostic criteria for idiopathic narcolepsy include (1) EDS occurring almost daily for at least three months or short sleep latency (SL) [less than 10 minutes (less than 8 minutes will be used in 2nd revision of ICSD)] by MSLT together with (2a) cataplexy (sudden and transient episodes of loss of muscle tone triggered by emotions; narcolepsy and with cataplexy in 2nd revision of ICSD) or (2b) with abnormal REM sleep features documented by polygraphic measures [more than two sleep onset REM periods (SOREMPs) in MSLT; narcolepsy without cataplexy in 2nd revision of ICSD].

In our review, symptomatic narcolepsy is defined as the cases that met the criteria (if MSLT data was not available, equivalent polygraphic REM sleep abnormalities were also considered, and this is noted for each case). In addition, association with a significant underlying neurological disorder accounts for the EDS and temporal associations (narcolepsy onset should be within 3 years if the causative diseases are "acute" neurologic conditions)(39). In contrast, if neither cataplexy nor polygraphic abnormal REM sleep features are associated with EDS (clinically "or" short sleep latency documented by polygraphic measures [typically less than 10 min (less than 8 min will be used in second revision of ICSD) during MSLT], the diagnosis of symptomatic EDS was made. In particular cases, such as EDS associated with ADEM, EDS may rapidly disappear with steroid treatments and may not last for 3 months. We diagnosed these cases arbitrarily as symptomatic EDS, and the duration of the EDS episode is noted.

Since the causal relationship between these two conditions were mostly judged by the statements of the authors' original case reports, it may be impossible to exclude cases in which the neurological condition is only a coexistence of idiopathic narcolepsy/idiopathic hypersomnia. In rare cases, isolated cataplexy (without EDS) associated with neurological conditions occurs. If the authors emphasize the occurrence of cataplexy as a significant underlying neurological disorder and no EDS is associated, we classified these as "symptomatic (isolated) cataplexy."

Using these criteria, we have counted about 116 symptomatic cases of narcolepsy reported in the literature [the details of all cases will be reported in our review article for symptomatic narcolepsy (40)].

As reported previously by several authors, tumors, inherited disorders, and head trauma are the three most frequent causes for symptomatic narcolepsy: 33 cases (29%) of symptomatic narcoleptic cases were due to brain tumors with 55% among them exhibiting cataplexy (29,41–60). The results of HLA typing were reported in 14 cases, and 8 cases were HLA DR2 negative. We also analyzed the brain structures involved in symptomatic cases of narcolepsy with brain tumors, and found that hypothalamic

lesions (70%) are most often associated. The brainstem lesions were much less frequent, found in only 10% of these cases. Other structures reported were 12% cases and 9% multiple sites.

Thirty-eight cases (34%) were due to inherited diseases, and 58% among them exhibited cataplexy. HLA typing was performed in 19 cases, and 11 of them were HLA negative. The lesions of inherited diseases were not specified by neuroimaging.

Nineteen cases (16%) were due to head trauma, and 74% among them exhibited cataplexy (21,27,39,53,61–69). In contrast to tumor cases, it is often difficult to determine the structure impaired for the symptomatic narcoleptic cases associated with head trauma, and there was no clear tendency regarding the brain structures responsible.

Ten cases (9%) of symptomatic narcolepsy studied were associated with multiple sclerosis (MS) (21,53,70–75). Most old cases were reported to exhibit both EDS and cataplexy, but many of them lack clinical details. It is thus difficult to exclude if some of these cases are only coexistence of idiopathic narcolepsy. HLA typing was performed in 4 cases, and 2 of them were HLA DR2 negative.

Six cases (5%) cases were due to vascular disorders. The lesions impaired were reported to be the hypothalamus ($n = 1$), thalamus ($n = 1$), brainstem (or both) ($n = 2$), or unspecified ($n = 2$). HLA typing was done in 4 cases, and 2 cases were HLA DR2 negative.

B. Anatomical Substrate for the Symptoms of Narcolepsy

Analysis of symptomatic narcolepsy with tumor cases clearly showed that the lesions were most often (about 70% of cases) involved in the hypothalamus and adjacent structures (the pituitary, supra seller or optic chasm). Von Economo (76) was probably the first person to suggest that narcolepsy may have its origins in the posterior hypothalamus and in some cases a secondary etiology. Neuropathological studies on encephalitis lethargica pandemic (1916–1923) revealed involvements of the midbrain periaqueducal grey matter and posterior hypothalamus in the hypersomnolent variant, with frequent extension to the oculomotor nuclei. This led von Economo to speculate that the anterior hypothalamus contained a sleep-promoting area while an area spanning from the posterior wall of the third ventricle to the third nerve was involved in actively promoting wakefulness. Along with von Economo's cases, two case reports for narcolepsy-cataplexy after encephalitis lethargica were also available by Stiefler (77) and Adie (17).

The cause of idiopathic narcolepsy was also speculated to involve this general area by von Economo (16). A postulated hypothalamic cause of narcolepsy was widespread until the 1940s (61), but was then ignored during the psychoanalytic boom (78,79), thereafter replaced by a brainstem hypothesis (80), along with establishments of the brainstem roles of generating REM sleep and REM sleep atonia (81). The involvement of the hypothalamus in occurrence narcoleptic symptoms was nicely refined by Aldrich et al. (29), who noted that tumors or other lesions located close to the third ventricle were associated with symptomatic narcolepsy and hypothesized that the posterior hypothalamic region may be the culprit. The hypothesis is finally confirmed by the discovery of hypocretin deficiency in idiopathic cases of human narcolepsy (3,82,83). The fact that impairments in the hypothalamus are noted in most symptomatic cases of narcolepsy also suggests a possible involvement of impaired hypocretin neurotransmission for this condition.

II. Hypocretin Status in Various Neurological Conditions

A. Hypocretin Status in Symptomatic Narcolepsy-Cataplexy Associated with Distinct CNS Lesions

Soon after the discovery of the involvement of hypocretin impairments in idiopathic narcolepsy, Melberg et al. (84) reported a reduced CSF hypoocretin-1 level (96 pg/ml) in a previously reported 51-year-old male case with autosomal dominant cerebellar ataxia (ADCA), deafness and narcolepsy (DN). In this Swedish pedigree (ADCA-DN; OMIM, Online Mendelian Inheritance in Man, accession number 604121), four out of five ADCA subjects are affected with narcolepsy-cataplexy (85), and CSF previously collected from one of theses subjects (patients III-2) was available for the hypocretin measures. The patient was negative for HLA-DR2. Since this case is a heredodegenerative disease with an enlargement of the third ventricle, moderate atrophy of the cerebellum and the cerebral hemispheres by MRI were observed, we listed this case under narcolepsy associated with distinct CNS lesions.

Scammell et al. (86) subsequently reported a 23-year-old male who developed narcolepsy-cataplexy due to a large hypothalamic stroke after a resection of a craniopharyngioma. This lesion included 2/3 of the caudal hypothalamus except for the most lateral component on the right, and extended into the mediodorsal thalamus bilaterally, the left amygdala, and parts of the basal forebrain and the rostral midbrain. His postoperative course was complicated by panhypopituitarism, staphylococcal meningitis, and hydrocephalus. He experienced HH. He became obese with a BMI of 31.7. Sleep latency by MSLT was 0.5 min and REM latency was 3.5 min. An overnight polysomnography showed 1 min and 1.5 min of SL and REM latency respectively without significant sleep apnea. His HLA was negative for DQB1*0602 and CSF hypocretin level was 167 pg/ml.

Nokura et al. (60) reported one case with narcolepsy and cataplexy-like phenomena, a 66-year-old female with hypersomnia due to a hypothalamic tumor. She showed EDS and cataplexy-like symptoms, such as an abrupt falling without loss of consciousness, but the emotional triggers were unclear. An MRI revealed lesions with high signal intensities in the hypothalamus, thalamus, and midbrain bilaterally (Fig. 1). This case was accompanied with mild anterior hypopituitarism and a SOREMP in a daytime polysomnography. Hypocretin-1 level was 61pg/ml. Her symptoms were improved with reduction of the tumor after 46 gray (Gy) radiation, and the intravenous administrations of nimustine hydrochloride and interferon beta.

The causes of the lesions in these three cases had different etiologies: degeneration, infarction, and tumor. Although the number of cases is still limited, the hypothalamic lesions were noted in all three cases. Moderate reduction of CSF hypocretin levels (2 low and 1 intermediate) also confirmed the functional impairment of the hypothalamus. It is likely that a massive impairment of hypocretin projections and projection sites are involved in the second case (hypothalamic stroke after a resection of a craniopharyngioma), implying that more severe impairment hypocretin neurotransmission (than that estimated from CSF hypocretin-1 level) may exist. Although these results are consistent with the hypothesis of the hypothalamic hypocretinergic involvement in symptomatic cases of narcolepsy, it is not certain if all cases with low hypocretin levels associated with hypothalamic damage develop narcoleptic symptoms.

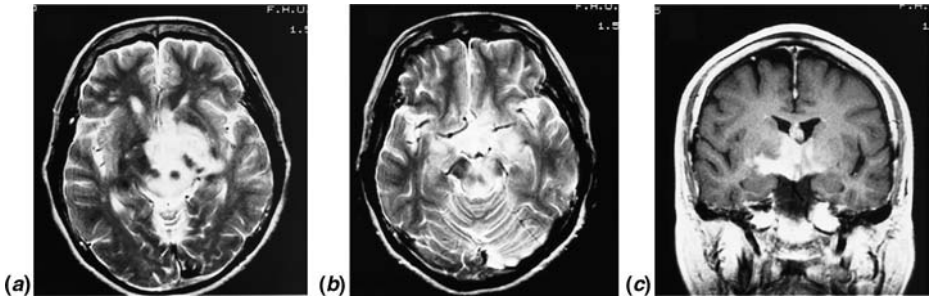


Figure 1 A narcolepsy-cataplexy case with hypothalamic tumor and low hypocretin level (61 pg/ml). A 66-year-old female with hypothalamic tumor. (***a***,***b***) Axial T2-weighted image of MRI at admission exhibits high signal intensities in the midbrain, hypothalamus, and thalamus. (***c***) Coronal T1-weighted image with gadolinium exhibits enhancement in the same lesion. This case also accompanied with mild anterior hypopituitarism. Her symptoms and MRI findings were improved with reduction of the tumor after 46 Gy radiation and nimustine hydrochloride and interferon beta were administered intravenously. *Source*: From Ref. 60.

B. Hypocretin Status in Symptomatic Narcolepsy-Cataplexy and/or EDS Associated with Inherited Disorders

There are clusters of cases of genetic or congenital disorders associated with primary central hypersomnolence and/or cataplexy, and CSF hypocretin-1 has also been assessed in several patients with PWS, NPC and myotonic dystrophy.

Prader-Willi Syndrome

Prader-Willi syndrome (PWS) is a genetically defined disorder that is characterized by infantile hypotonia and failure to thrive, hyperphagia with early childhood obesity, hypogonadism, temperature instability and developmental delay (87–89). The molecular genetic cause is the nonexpression of the paternal genes in the PWS region on chromosome 15q11-13 (90).

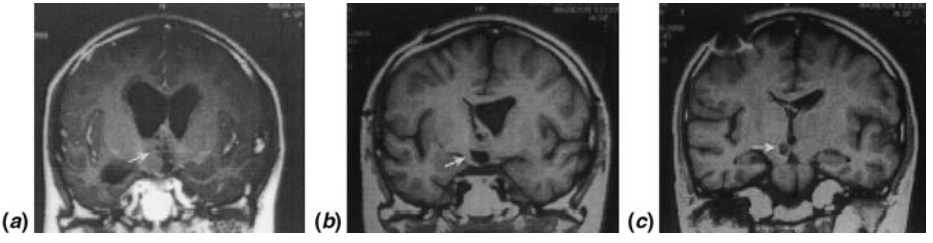


Figure 2 A hypersomnia case with hypothalamic tumor and low hypocretin level (104 pg/ml). Preoperative coronal MR image. In (***a***), a suprasellar mass (*arrow*) fills the mid and posterior hypothalamus, compressing the third ventricle. In (***b***) and (***c***) postoperative coronal T1-weighted MR images, the bilateral, medial, and lateral hypothalamic areas (***b***, *arrow*) and right posterior hypothalamus (***c***, *arrow*) are damaged. *Source*: From Ref. 60.

EDS is a common symptom in PWS (87–89). Sleep disordered breathing (SDB) and narcoleptic traits such as SOREMPs and cataplexy have also been reported in these subjects (90,91). If SDB exists, primary hypersomnia should only be diagnosed if excessive daytime sleepiness does not improve after adequate treatment of SDB. Mignot and Nevsimalova et al. (2) reported a 16 year old male with EDS, HLA-DQB1*0602 positive, obese (BMI = 48.1), with documented 15q11-13 deletion, limited number of SDB events [apnea hypoxia index (AHI) was 5.6], and no cataplexy; SL = 3.0 min, no SOREMPs by MSLT and hypocretin 109 pg/ml. Nevsimalova et al. (92) also measured CSF hypocretin-1 in another three PWS cases with one of subject (10y) exhibited EDS (SL = 6.0 min) with no SOREMPs and AHI is 3.1. All three subjects were obese and exhibited no cataplexy. CSF hypocretin levels in the PWS case with EDS and DQB1 *0602 was low (130 pg/ml, 10y, BMI = 29.8, AHI = 3.1) and others without EDS are intermediate (191 pg/ ml, 23y, BMI = 49, AHI = 46.8) or in the normal range (226 pg/ml, 6y, BMI = 25.8, AHI = 0). Interestingly, AHI in these PWS subjects are correlated with age and body mass index (BMI), but not with CSF hypocretin-1 levels and EDS.

Arii et al. (15) reported a two week-old PWS male with severe hypotonia, poor feeding, documented 15q11-12 deletion and hypocretin was intermediate (192 pg/ml).

These reports raised the possibility that EDS in PWS may also be attributed to the hypocretin system, not to sleep disordered breathing caused by obesity. The latter author also proposed that PWS cases may be a model for congenital dysfunction/ developmental failure of the hypocretin system.

Niemann–Pick Type C Disease

NPC is an autosomal recessive and congenital neurological disorder characterized by the accumulation of cholesterol and glycosphingolipids in the peripheral tissues and of the glycosphingolipids in the brain. Classic NPC symptoms include hepatosplenomegaly, vertical supranuclear gaze palsy, ataxia, dystonia, and dementia. Subjects with NPC have been reported to frequently display narcolepsy-like symptoms, including cataplexy (18,28,93–95). This condition is remarkable as cataplexy is often triggered by typical emotions (laughing) and responsive to anticataplectic treatments.

Kanbayashi et al. (95) measured CSF hypocretin levels in two NPC cases with and without cataplexy. The first case was a five-year-old boy with NPC, cataplexy and an intermediate hypocretin level (142 pg/ml). Cataplexy was evoked by laughter since the age of 2.3 years. EDS was not claimed by the patient, and normal SL (16.5 min) without SOREMPs was observed by a two-nap sleep test (TNST) (96). No abnormal findings in the hypothalamus were detected by MRI scans. He was negative for HLA DR2. The second case was a three-year-old girl with NPC with a normal hypocretin level (299 pg/ml). This patient exhibited neurological symptoms such as tremor, ataxia and akathisia, but did not exhibit cataplexy or EDS.

Vankova et al. (93) reported five patients with juvenile NPC. Deterioration of intellectual function; the presence of pyramidal, dystonic and cerebellar signs, and splenomegaly were observed in all cases. Cataplexy was reported in one patient. Nocturnal polysomnography revealed disrupted sleep in all patients. Total sleep time, sleep efficiency, REM sleep, and delta sleep amounts were decreased when compared to

age-matched controls. Shortened mean sleep latencies were observed in three patients during the MSLT, but SOREMPs were observed only in the case with cataplexy, and this case met with the criteria of symptomatic cases of narcolepsy. This patient was HLA DQB1*0602 positive, while the other subjects were HLA DQB1*0602 negative. CSF hypocretin-1 levels were reduced in patients (190 pg/ml and 157 pg/ml in 1 with cataplexy) while in the two other patients, the CSF hypocretin-1 were at the lower level (226 pg/ml, 245 pg/ml) of the normal range. The authors speculated that lysozomal storage abnormalities in NPC patients may also have the impact on the hypothalamus including, area hypocretin-containing cells are located.

In these two reports, all of the NPC patients with cataplexy have an association with reduced hypocretin-1 levels, while CSF hypocretin-1 levels in the NPC cases without cataplexy are in the lower limit of normal, suggesting a degree of impairments of the hypocretin system may contribute the occurrence of cataplexy in this inherited diffuse CNS impairment condition.

Myotonic Dystrophy

Myotonic dystrophy type 1 (MYD1) is a multisystem disorder with myotonia, muscle weakness, cataracts, endocrine dysfunction, and intellectual impairment (97–99). This disorder is caused by a CTG triplet expansion in the 3′ untranslated region of the DMPK gene on 19q13. MYD1 is frequently associated with EDS, sharing with narcolepsy a short sleep latency and the presence of SOREMPs during the MSLT. The disease is also often associated with SDB, and thus this may also count for appearances of SOREMPs. Martinez-Rodriguez (20) reported six patients with MYD1 complaining of EDS. The mean sleep latency on MSLTs was abnormal in all patients ($<$5 min in 2, $<$8 min in 4) and two SOREMPs were observed in two subjects, being met with the criteria for symptomatic narcolepsy. It should be noted that these two cases also had SDB. All patients were HLA-DQB1*0602 negative. Hypocretin-1 levels (181 pg/ml) were significantly lower in patients versus controls (340 pg/ml); one case with two SOREMPs had hypocretin-1 levels in the range generally observed in narcolepsy ($<$110 pg/ml). Three cases had intermediate levels (110–200 pg/ml). The authors suggested that a dysfunction of the hypothalamic hypocretin system may mediate sleepiness and abnormal MSLT results in patients with MYD1.

In one case of late-onset congenital hypoventilation syndrome, a disorder with reported hypothalamic abnormalities (100), Martinez-Rodriguez found very low CSF hypocretin-1 levels in an individual with otherwise unexplained sleepiness and cataplexy-like episodes (20). Excellent response to anti-cataplectic medication was observed in this case.

Although only a limited number of cases with genetic or congenital neurologic conditions associated with EDS and/or cataplexy was studied, moderate decreases in CSF hypocretin levels was observed in almost all cases with EDS and/or cataplexy. However, the degree of reduction was small in contrast to idiopathic narcolepsy-cataplexy. Moreover, CSF hypocretin-1 levels in other genetic and congenital neurological condition without EDS/cataplexy are not systematically studied, it is still uncertain for the specificity of impaired hypocretin system in EDS and cataplexy in these above mentioned neurological conditions.

III. Hypocretin Status in Hypersomnia in Various Neurological Conditions

A. Focal/Generalized CNS Invasion

Symptomatic narcolepsy is relatively rare, but sleepiness without other narcoleptic symptoms can often occur with a variety of neurological disorders; they are more likely to be due to multifocal or global disturbances of the brainstem, diencephalon and cerebral cortex. Recently, several clinical studies also suggested that the disruption of the hypothalamic hypocretin system in EDS associated with various neurological conditions.

Cerebral Tumors

Arii et al. (57) reported a case with hypersomnia in a 16-year-old female after removal of a hypothalamic suprasellar Grade II pilocystic astrocytoma. MRI showed the bilateral, medial, and lateral hypothalamic areas and right posterior hypothalamus were damaged (Fig. 2). This case was accompanied with DI, hypothyroidism, weight gain, no cataplexy, MSLT: sleep latency: 1.7 min, no SOREMPs, HLA-DR2 negative and hypocretin-1 104 pg/ml.

Marcus et al. (54,101) reported an 11-year-old boy in a vegetable state following astrocytoma resection and CNS hemorrhage. An MRI revealed a large suprasellar mass that extended into the sella inferiorly and was displaced posteriorly. The boy developed hypothyroidism and syndrome of inappropriate antidiuretic hormone (SIADH). In the nocturnal EEG study, sleep was fragmented with 16 short REM cycles. The daytime EEG showed frequent REM periods. HLA-DR2 and DQB1*0602 was negative. Hypocretin-1 was undetectably low level. His EDS had improved with 200 mg modafinil and 5 mg methylphenidate.

Snow et al. (55) reported that five patients (11–19 years, mean: 15 years) with EDS. The mean sleep latency by MSLT in the five patients was 10.3 min, but no detailed sleep data was reported for each case. Three patients underwent surgeries for craniopharyngioma, one for germ cell tumor, and one for a thalamic arachnoid cyst. The craniopharyngiomas and germ cell tumor were located in the hypothalamus-hypophysis region, and the arachnoid cyst was in the thalamic region. All patients received relatively extensive surgeries involving the hypophysis and hypothalamus and hormone replacement therapies. Patients had significantly higher BMI (mean: 28), and this was primarily attributable to 2 morbidly obese patients associated with obstructive sleep apnea. Although treatment with continuous positive airway pressure resulted in complete resolution of their SDB in these two cases, no changes in daytime somnolence occurred.

Krahn (58) reported a patient who developed a narcoleptic-like sleep disorder after receiving treatment for a choroid plexus carcinoma of the pineal gland. She underwent a pinealectomy, chemotherapy, and radiation treatment. Immediately after surgery, the patient developed EDS that she attributed to severe insomnia and an irregular sleep/wake rhythm. She had a few episodes of SP and HH but no cataplexy. An increased percentage of REM sleep was seen in nocturnal polysomnography, and three out of four SOREMPs were seen during the MSLT. She was negative for HLA-DQB1*0602 and had a normal cerebrospinal fluid hypocretin level (518 pg/ml). The author proposed that her symptoms be caused by an unknown mechanism unrelated to hypocretin depletion.

Dempsey et al. (59) reported a case of 60-year-old male with acromegaly, who developed narcolepsy-cataplexy two weeks after completing radiotherapy (45 Gy) for a pituitary adenoma. He had both HH and SP. Sleep latency by MSLT was 6.4 min and REM latency was 9 min (3 SOREMPs/5 naps). He was obese (BMI: 35) and his AHI was 17/hour. HLA was not typical for narcolepsy. Hypocretin-1 was within normal range (275 pg/ml). The authors have speculated that the radiotherapy or the tumor was associated with damage to a locus rich in hypocretin receptors. In contrast to the case with Nokura et al. (60), the same 45–46 Gy radiation resulted in the opposite outcomes.

Kubota et al. (102) reported one typical case of narcolepsy–cataplexy with a ganglioma in the right amygdala in a 7-year-old girl. She showed hypnagogic hallucinations and a SOREMP in the nocturnal polysomnography. Sleep latency by MSLT was 6.5 min without SOREMP. Her HLA was DR2/DQw1 and hypocretin-1 level was 79 pg/ml. This case is likely to be the comorbidity of idiopathic narcolepsy and a brain tumor, since her symptoms were not changed after the resection of the tumor. This case is not listed in Table 1.

Overall, three symptomatic cases with EDS had low hypocretin-1 levels, however two other cases and Snow's five cases had normal levels. It should be noted that all three cases with low CSF hypocretin-1 levels are HLA-DR2 or HLA-DR2 and DQB1*0602 negative. Therefore, EDS in these HLA negative cases are likely to be secondary due to the hypocretin deficiency being caused by the tumors. EDS in the remaining seven cases with normal or high hypocretin-1 levels were thought to be caused by other factors, although there is also a possibility of impaired hypocretin projections, terminals or postsynaptic receptors caused by the tumors in these cases.

Infarctions

Bassetti et al. (8) reported two cases with EDS and cerebral infarction. The first case was a 34-year-old male. He suffered from thalamic infarction and his mean sleep latency by MSLT was 9 min. His hypocretin level was 265 pg/ml. The second case was a 40-year-old male who suffered ponto-medullary infarction. His sleep latency by MSLT was 1 min and hypocretin level was 316 pg/ml.

Nokura et al. (60) and Tohyama et al. (103) independently reported two hypersomnia cases with bilateral paramedian thalamic infarctions. The paramedian thalamus believed to play an important role in the regulation of sleep, and disturbances of sleep regulation are known to occur in paramedian thalamic stroke (104, 105). The first case was a 45-year-old male (60). He suffered from bilateral paramedian thalamic infarctions and had EDS with SOREMPs (two times in four naps) by MSLT (met with the criteria for symptomatic narcolepsy). His hypocretin-1 level was 312 pg/ml. The second case was a 15-year-old male who suffered from bilateral paramedian thalamic infarctions and hypersomnia. His hypocretin level was 274 pg/ml (103). Since the lesions of infarctions did not include the hypocretin cell bodies, their hypocretin levels seemed to be normal. However, impairments of hypocretin projection still could be involved. It should be also noted that Guilluminault et al. (104), pointed out that patients with bilateral paramedian thalamic lesions do not present a typical hypersomnia but a de-arousal or subwakefullness with an inability to develop sleep outside the normal circadian boundary (pseudo-hypersomnia). Indeed these patients

showed reduced latency to stage 1 during MSLT, but did not develop other normal non REM sleep and REM sleep status during daytime. It may also be possible that hypocretin deficiency is not involved in so-called pseudo-hypersomnia associated with bilateral paramedian thalamic lesions, and other pathophysiology needs to be considered for these unique sleep symptoms.

Encephalopathies

Wernicke's Encephalopathy

Kashiwagi et al. (106) reported a five-year-old girl with Wernicke's Encephalopathy (Fig. 3). She gradually developed sleepiness and an abnormal sleep/wake schedule. Her sleep time was 15 to 20 hours per day and fell asleep frequently even while eating. She developed ocular symptoms and neurological symptoms (such as involuntary movements, hemiparesis, depression of speech and global confusional state). An MRI revealed lesions in the bilateral hypothalamus in addition to the dorsomedial nucleus of thalamus and mammillary bodies and periaqueductal gray and floor of 4th ventricle. Vitamin B1 levels were low (38.7 ng/ml, normal range: 52–176 ng/ml) and the level of hypocretin of CSF was decreased (<40 pg/ml). Her sleepiness and MRI findings gradually improved with thiamine therapy. Six months after

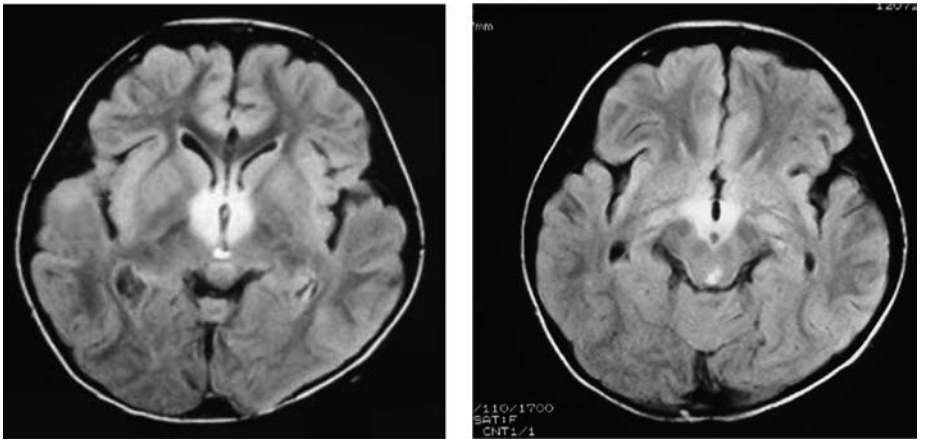


Figure 3 A hypersomnia case with hypothalamic lesion due to Wernicke's encephalopathy and undetectable hypocretin level. Kashiwagi et al. reported a 5-year-old girl with Wernicke's encephalopathy. She gradually developed sleepiness and an abnormal sleep/wake schedule. Her sleep time was 15-20 hours per day and she fell asleep frequently even while eating. She developed ocular symptoms and neurological symptoms (such as involuntary movements and hemiparesis and depression of speech and global confusional state). MRI revealed lesions in bilateral hypothalamus in addition to dorso-medial nucleus of thalamus and mammillary bodies and periaqueductal gray and floor of IVth ventricle. The level of vitamin B1 was low. The level of hypocretin was decreased (<40 pg/ml). Her sleepiness and MRI findings gradually improved with replacement of vitamin B1. Six months after the onset of sleepiness, both MRI lesion and CSF hypocretin level (158 pg/ml) recovered to some degree. *Source*: From Ref. 106.

the onset of sleepiness, both MRI lesion and CSF hypocretin level (158 pg/ml) recovered to some degree. Although is likely that brain lesions in the cases with tumors, Wernicke's encephalopathy affect the hypothalamic hypocretin system directly or indirectly, it is not fully studied whether the change in the hypocretin neurotransmission is solely responsible for the occurrence of the EDS in these cases.

Limbic Encephalopathy

Yamato et al. (107) reported a patient with non-paraneoplastic immune-mediated limbic encephalitis exhibiting low hypocretin-1 concentrations (87 pg/ml). A 65-year-old male developed chronic progressive hypersomnia. An MRI of the brain showed bilateral signal abnormalities in the medial temporal lobes and the hypothalamus, but systemic examinations for malignant tumors were negative. Acyclovir treatment failed to amend his condition. Subsequent steroid treatment improved his hypersomnia and reduced the extent of abnormal signals on MRI. The CSF hypocretin concentration increased to 148 pg/ml in 23 days after.

Rasmussen's Syndrome

Lagrange et al. (108) reported a case of narcolepsy and Rasmussen's syndrome in a previously healthy 40-year-old man. He developed severe EDS, cataplexy, HH, and SP over the course of a few months. Brain MRI was normal and polysomnography with MSLT confirmed a diagnosis of narcolepsy (SL: 1.6 min, three SOREMPs in four naps). His HLA haplotype is DQB1*0602, and CSF analysis showed no detectable hypocretin. Approximately 18 months later, he developed complex partial seizures. Further MRI showed a progressively enlarging lesion involving the left frontotemporal and insular areas. Pathology from a partial resection samples was consistent with Rasmussen's syndrome. Evaluation for tumor, infectious, and paraneoplastic etiologies was negative. There was no further progression of the residual lesion on serial MRI.

Although the pathophysiological bases of narcolepsy and Rasmussen's syndrome are unknown, the author speculated the possibility of a common underlying disease processes related to autoimmune mechanism. It is however, a temporal relationship between hypocretin deficiency and the onset of the disease in this case that is not known. It may also be possible for the comorbidity with idiopathic narcolepsy, since the subjects is HLA positive, and late onset cases of idiopathic narcolepsy are also reported.

Neurodegenerative Disorders

Parkinson's Disease

Thirty percent of patients with Parkinson's disease (PD) have been reported to have EDS. Sleep problems are often related to the disease itself (e.g., difficulties in maintaining sleep because of motor disabilities), but they can also occur secondary to pharmacological treatment especially with dopamine D2/3 agonists. Ripley et al. (13) initially reported that CSF hypocretin-1 in 7 PD subjects were in the normal range, but sleep abnormalities of these subjects were not assessed. Overeem et al. (109) measured CSF hypocretin levels in three PD patients with EDS, and all had normal hypocretin-1 levels.

Drouot et al. (110) reported that patients with late-stage PD had low ventricular CSF hypocretin-1 levels ($n = 16$: <50–97 pg/ml, $N = 3$: 138–169 pg/ml). Hypocretin-1 levels decreased with increasing disease severity. The author described that

CSF hypocretin-1 levels may reflect the size of the hypocretin neuron pool, and a decrease in hypocretin-1 levels may indicate degeneration of hypocretin neurons in PD. The sleepiness of the patients was assessed by Epworth sleepiness scale (ESS). The mean ESS of these PD patients (11 ± 1) was significantly higher than that of controls (4 ± 1), but hypocretin-1 level was not correlated with ESS among PD subjects. The discrepancy between this study and Overeem's has not been determined yet.

Dementia with Lewy Bodies

The dementia with Lewy bodies (DLB) is the second major type of senile, degenerative dementia, after the AD. DLB shares many features with PD. EDS, hallucinations and REM sleep behavior disorder are symptoms reported in both DLB and narcolepsy. However, Baumann et al. (111) reported that patients with DLB had normal hypocretin levels.

Progressive Supranuclear Palsy

Hattori et al. (112) reported a 74-year-old woman with EDS who was diagnosed as probable progressive supranuclear palsy (PSP). Her EDS mimicked narcolepsy without cataplexy, because MSLT showed short latencies (less than 2 min without SOREMPs), HLA was positive for DR2/DQB1 and CSF hypocretin-1 concentration was undetectable. It is not clear that the coincidence of these disorders is due to a common process or comorbidity. The author speculated that the existence of neuropathological changes, such as neurofibrillary tangles in hypothalamus of the patient with PSP might cause decreased hypocretin neurotransmission.

Alzheimer's Disease

Riply et al. (13) also reported that CSF hypocretin-1 levels in 24 patients with Alzheimer's disease (AD) were normal. This condition was known with established sleep abnormalities (113). Dysfunction of other neurochemical systems, for example cholinergic systems in AD, may be more directly involved in sleep abnormalities in these subjects.

Head Trauma

The association of narcolepsy/EDS with head injury is controversial. Most people with hypersomnolence after closed head injury do not have narcolepsy (62), but some patients with narcolepsy report that their symptoms began after a head injury (39,63–66,69). Lankford et al. (39) reported 9 detailed cases with narcolepsy (5 HLA positive, 2 HLA negative, and 2 undetermined). However, these cases lacked hypocretin-1 measurements. Riply et al. (13) reported decreased CSF hypocretin-1 levels (5 out of 6 cases) after the head trauma.

Dauvilliers et al. (67) reported that the patient severely affected with post-traumatic hypersomnia with brain lesions (as determined by MRI) had an intermediate CSF hypocretin-1 level (176 pg/ml, HLA negative), while the next severely affected patient had a normal level (503 pg/ml, HLA positive). These two patients had no cataplexy but had shortened sleep latencies (4.5 min, 3.0 min, respectively) without SOREMPs by MSLT.

Arii et al. (68) reported a 15-year-old male affected with post-traumatic hypersomnia with an intermediate hypocretin-1 level. His Glasgow scale at 48 hours after injury was 12 (E2V4M6). An MRI showed severe cerebral contusion of the bilateral basalis of the fronto-temporal lobe and medial part of right occipital lobe with CSF leakage. One year after injury, he needed more than nine hours nocturnal sleep and one or two 1–3 hours naps daily. The hypocretin-1 level was 151 pg/ml. MRI showed atrophies in the basalis of temporal lobe and medial part of right occipital lobe. The hypothalamus showed moderate atrophy with dilatation of third ventricle but no localized lesion.

EDS appearing during the first year following a head injury may be considered as post-traumatic (114). This typically presents itself as extended night sleep and episodes of daytime sleep. Sleepiness is usually associated with other characteristics such as headaches, difficulties in concentration or memory disorder. Radioimaging studies may reveal several possibilities: lesions affecting the hypothalamic region or brainstem, midbrain or pontine tegmentum, or more often than not, the absence of any significant lesions. Sleepiness should be objectively evaluated by a MSLT but is often is not in clinical situation. Cases with hypersomnia after head or brain trauma associated with sleep apnea syndrome were also reported (62).

Although two out of three patients with post-traumatic EDS had decreased CSF hypocretin-1 levels moderately, it is not known whether all post-traumatic subjects with declined CSF hypocretin-1 levels exhibit EDS. Similarly, it has not been studied whether more pronounced degree of hypocretin-1 impairments is evident for the post-traumatic symptomatic narcolepsy.

B. CNS Diseases Mediated with Neuroimmune Mechanisms

As mentioned earlier, 10 cases of narcolepsy-cataplexy are reported in MS (21,53, 70–75). The question was also raised as to whether there is a fortuitous coexistence of the two disorders or whether a causal relationship exists between them. Some cases with late onset and regressive course suggested demyelination as the course of narcolepsy, but some authors also suggested an involvement a common genetic susceptibility (i.e., HLA DR2) (115). Among these previous cases, a case reported by Younger et al. (73) had a lesion in cerebral peduncles detected by MRI, but specific lesions were not noted for a majority of other cases. Recently, three EDS cases associated with MS (60,74,75,116) with hypothalamic lesion and reduced hypocretin-1 levels suggest an involvement of the hypothalamus in MS associated with EDS/cataplexy. EDS in four ADEM cases were recently reported (117–120). All these cases associated EDS had hypothalamic lesions and low CSF hypocretin-1 levels, suggesting of the hypothalamic hypocretin system in these conditions.

Demyelinating Diseases

Multiple Sclerosis

Iseki et al. (74,75) reported a 22-year-old female case of MS presenting with hypersomnia and several SOREMPs secondary to bilateral hypothalamic lesions. Her nocturnal sleep time was 15 hours and sleep latency by MSLT was 2.8 min and REM latency was 4.7 min (5 SOREMPs in 5 naps). She did not experience cataplexy, HH, or SP. Her HLA was DR4 and DR6. An MRI revealed fluid attenuated inversion recovery (FLAIR) hyperintensity in the hypothalamus bilaterally and CSF hypocretin-1

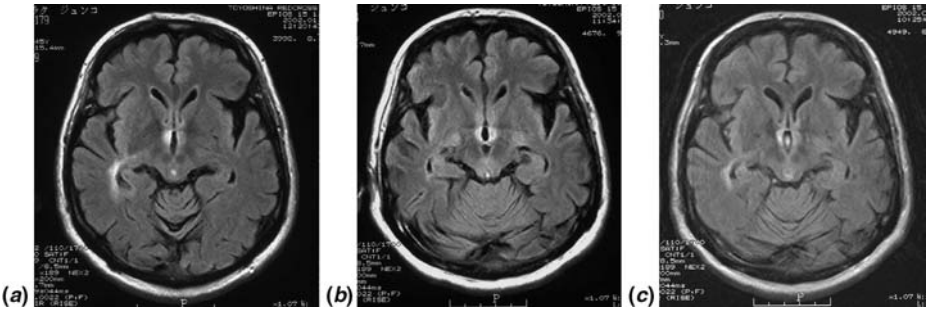


Figure 4 A MS case with hypersomnia and undetectable hypocretin level. Kato et al. (116) reported a 45-year-old female with manifested hypersomnia in a relapse of MS. (***a***) One-half month before occurrence of hypersomnia. There was only a right hypothalamic lesion. (***b***) 3rd hospital day: The patient was suffering from hypersomnia at this time. There was a newly occurred lesion of left hypothalamus. (***c***) 32th hospital day: Hypersomnia had already subsided. It is noteworthy that the left hypothalamic lesion disappeared. From this case, the bilateral lesions will be needed for the symptom of hypersomnia.

level was <40 pg/ml. After intravenous methylprednisolone was started and followed by oral predonisolone treatment, her symptoms resolved and MRI findings were improved. The hypocretin-1 levels improved to 167 pg/ml after two month and 221 pg/ml after 4 months.

Kato et al. (116) reported a 45-year-old female with manifested hypersomnia in a relapse of MS (Fig. 4). Five days before admission, she suffered from hypersomnia; she suddenly fell asleep during a conversation and again while cooking. After the admission, she slept almost all day. MRI revealed T2 and FLAIR high lesion with hypothalamus bilaterally. Hypocretin-1 level in CSF was below 40 pg/ml. Methylprednisolone pulse treatment was started, followed by oral prednisolone treatment. Three days after the initiation of methylprednisolone, her hypersomnia was completely resolved. Twenty days later, hypocretin-1 levels recovered to 167 pg/ml.

Nozaki et al. (121) reported a 43-year-old female case MS presenting with a period of hypersomnia and febrile. MRI presented T2 high lesion of hypothalamus bilaterally. CSF hypocretin-1 level was 191 pg/ml. Intravenous methylprednisolone was started and was followed by oral predonisolone treatment; her symptoms resolved and MRI findings improved. The level of hypocretin-1 was increased to 291 pg/ml.

Although all earlier symptomatic narcolepsy associated with MS cases showed cataplexy, three recent MS cases with low hypocretin levels (<40 and 191 pg/ml) did not exhibit cataplexy or REM sleep abnormalities. In these cases, extended nocturnal sleep as well as hypersomnia was observed. Since diagnostic methods and therapies for MS have improved, especially treatments using steroid, interferon, intravenous immunoglobulins (IVIg) and other immunosuppressants are common, early interventions possibly lead the acute relapse phases to the rapid remissions. These situations may possibly change the symptomatology of sleep abnormalities associated with these MS cases that have been recently reported. Interestingly, in each of these three reported MS cases, CSF hypocretin levels increased and the sleep abnormalities subsided in

relatively short period with the steroid treatments. In other words, a chronic impairment of hypocretin neurotransmission may be required to exhibit cataplexy. Roles of HLA in occurrence of cataplexy in these cases were not evaluated, due to the limited number of cases (and the availability of HLA data). Findings observed in these recent MS cases may also be informative for developing new treatments for idiopathic cases of narcolepsy. The combined observation of hypocretin deficiency (possibly due to hypocretin cell loss) and HLA association suggests an autoimmune basis for idiopathic cases of narcolepsy. If this is the case, the process may also be reversible prior to or near complete ablation of cells. Based on this hypothesis, steroid treatment and/or intravenous immunoglobulins (IVIg) have been tried in several hypocretin deficient young narcoleptic subjects. Although small beneficial effects were reported in some of these cases (see Ref. 122 for details), the assessments are subjective, and further evaluations (possibly using proper double blind, placebo- controlled trials) are needed.

Acute Disseminated Encephalomyelitis

Kubota et al. (117) reported a 12 year-old girl with acute disseminated encephalomyelitis (ADEM) and hypersomnia. An MRI revealed lesions in bilateral hypothalamus in addition to other multifocal lesions including cerebral white matter, brain stem and basal ganglia. MSLT revealed mean sleep latency was 4.5 minutes with no sleep-onset REM periods. HLA typing of this patient was negative for DQB1*0602. The CSF hypocretin-1 was 102 pg/ml. Her hypersomnia and MRI findings including in hypothalamus and the other regions improved with the steroid treatment.

Gledhill et al. (118) reported a 38-year-old female with ADEM and hypersomnia. She had no REM related symptoms, such as cataplexy, HH, or SP. An MRI revealed lesions in the hypothalamus, walls of 3rd ventricle, corona radiata, floor of the aqueduct and raphe nuclei. She was positive for DR2/DQB1*0602. Hypocretin-1 levels were 87 pg/ml. She was treated with a high dose steroid and a subsequent MRI showed smaller and fewer lesions. Six months after, her subjective sleepiness was partially improved. Her mean sleep latency by MSLT (4 naps) was 4.4 min with 4 SOREMPs, and hypocretin-1 level was 148 pg/ml at this time point. One year after her initial examination, her sleepiness persisted and the results of MSLT were almost unchanged.

Yoshikawa et al. (119) reported a 7-year-old girl with ADEM, visual symptoms and hypersomnia (Fig. 5). An MRI revealed bilateral lesions in the white matter, basal ganglia, and hypothalamus. Her CSF hypocretin-1 levels were intermediate (146 pg/ml) on admission, gradually recovered to the normal range (263 pg/ml) during 47 days, and her excessive sleepiness was reduced. Decreased hypothalamic hypocretin neurotransmission may be involved in this symptomatic case of hypersomnia associated with clinical course of ADEM. Interestingly, double vision was also noted in this case during their course of the disease.

Yano et al. (120) reported an 11-month-old girl with ADEM and hypersomnia. An MRI showed multiple T2 high intensity lesions in the white matter, brainstem, and bilateral hypothalamus. CSF hypocretin-1 was at undetectable levels. Hypersomnia and MRI lesions were improved by intravenous steroid administration. Changes in CSF hypocretin levels were not monitored.

EDS can be associated with immunological or post-infectious brain pathology, such as ADEM and other encephalitis. Von Economo's reports suggested that the

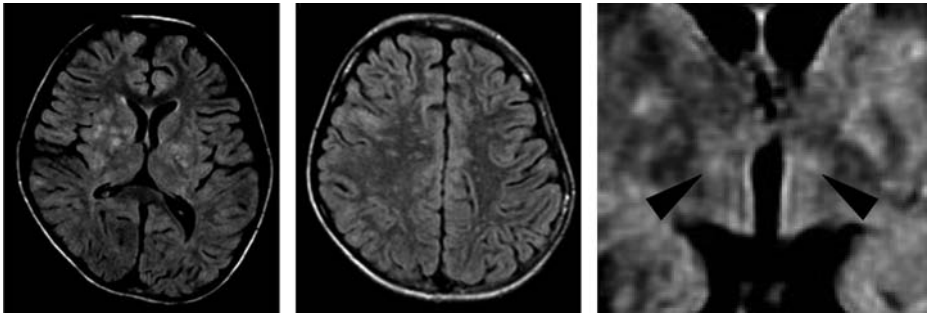


Figure 5 An ADEM case with EDS, visual symptoms and intermediate hypocretin level (146 pg/ml). Yoshikawa et al. (119) reported a 7-year-old girl with ADEM, visual symptoms and hypersomnia. MRI revealed bilateral lesions in the white matter, basal ganglia, and hypothalamus. Her CSF hypocretin level was intermediate (146 pg/ml) on admission, which gradually recovered to the normal range (263 pg/ml) during 47 days, as her excessive sleepiness was reduced.

hypothalamus is a target site for immune-mediated post infectious disease (76). An immunologic reaction to hypothalamic antigens, including the hypocretin system, may also be involved in immune-mediated encephalitis, however, after a series of von Economo's publications, relatively few cases of secondary EDS associated with post viral infection were reported. In recent several years, four ADEM cases with hypocretin measurements were reported. Although a decreased level of consciousness is frequently shown in cases with ADEM, ADEM cases with well defined hypersomnia are relatively rare (123,124).These cases show both extended nocturnal sleep time and daytime hypersomnia, and only one case shows SOREMPs, indicating sleep abnormalities may be distinct from those seen in symptomatic narcolepsy and idiopathic narcolepsy. Early diagnosis and treatment in recent cases may also reduce the severity of sleep symptoms. As discussed in MS section, early diagnosis and initiation of the steroid treatment may possibly change the symptomatology of sleep abnormalities associated with ADEM cases. EDS in these cases may be transient and be less than 3 months, and this may need attention since EDS more than 3 month are required for the diagnosis of the symptomatic cases of narcolepsy in the second revision of ICSD ("Narcolepsy associated with a known physiological condition" in the 2nd revision of ICSD).

Guillain-Barré Syndrome

Undetectably low CSF hypocretin-1 levels were found in seven cases of Guillain-Barré syndrome (GBS) in the Japanese population (13,125,126). Reduced CSF hypocretiun-1 levels in GBS are not likely to due to the increase in the protein in the CSF nor to the secondary effects due to the treatment or associated health conditions, but might be under influence of region/ethnicity-specific form of GBS (127). This finding was a rather unexpected, since GBS is a presumed autoimmune disorder of the peripheral polyradiculoneuropathy. However, additional CNS involvements (i.e., hypothalamus), such as occurrence of SIADH and DI, has also been suggested in severe cases.

Interestingly, all these GBS subjects with low hypocretin-1 were severe cases and had tetraplegia, bulbar symptoms, and/or respiratory failure.

Since the clinical picture of these subjects is quite different from narcolepsy, it is unlikely that there will be any diagnostic confusion by CSF hypocretin-1 levels. The occurrence of sleep abnormalities in GBS, especially in severe cases, has received little attention. The sleep latency for two CSF hypocretin-deficient GBS subjects who claimed sleepiness after the recovery of GBS neurological symptoms were significantly shortened (less than 1 minute) were in both cases (126).

Paraneoplastic Syndrome

Anti-Ma2 associated encephalitis. A recent report described four patients with paraneoplastic anti-Ma2 antibodies who had hypothalamic inflammation, EDS and undetectable CSF hypocretin-1 levels (128). Interestingly, CSF hypocretin levels in two other patients with paraneoplastic anti-Ma2 antibodies who did not exhibit EDS, were in the normal range (128). The MRI showed abnormalities involving medial temporal lobes, hypothalamus, basal ganglia or upper brainstem in these four patients. In addition, one case also had DI and hypothyroidism. The author claimed that anti-Ma associated encephalitis is the identified immune mediated disorder of the CNS that may result in low hypocretin-1 levels.

In contrast to MS and ADEM, distinct CNS lesions are not observed in GBS and neoplastic syndromes. Nevertheless, a significant degree (undetectable level) of hypocretin deficiency was observed in both conditions. This suggests that a hypocretin deficiency in these conditions may occur at the neuron or ligand levels. In view of the fact that an autoimmune hypothesis is the most popular theory for hypocretin cell death in narcolepsy (129,130), but no gloss inflammation was observed in the hypothalamus (82), a subset of GBS and Ma2 antibody positive paraneoplastic syndromes (the two neuroimmune conditions associated with hypocretin deficiency), may be important models for studying a possible autoimmune cell damage in narcolepsy.

IV. Conclusion

Symptoms of narcolepsy can occur during the course of neurological conditions. Although it is difficult to rule out the comorbility of idiopathic narcolepsy in some cases, the review of literature reveals numerous unquestionable cases with symptomatic narcolepsy. These include cases with HLA negative and/or late onset, and cases in which the occurrences of the narcoleptic symptoms are parallel with the rise and fall of the causative disease. Symptomatic cases of narcolepsy are most often associated with brain tumors and inherited disease followed by head trauma. Cases associated with vascular diseases, degeneration and autoimmune/immune-mediated diseases are also reported. Review of these cases, especially with brain tumors, illustrates a clear picture that the hypothalamus is most often involved. Several cases of symptomatic cataplexy (without EDS) are also reported. In contrast, symptomatic cataplexy appeared to be often associated with non-hypothalamic structures.

Recently, it was revealed that the pathophysiology of idiopathic narcolepsy was linked to hypocretin ligand deficiency. CSF hypocretin-1 measures were also carried out in a limited number of symptomatic cases of narcolepsy/EDS. Reduced CSF

hypocretin-1 levels were seen in most symptomatic cases of narcolepsy/EDS with various etiologies, and EDS in these cases is sometimes reversible with an improvement of the causative neurological disorder, and also an improvement of the hypocretin status. It is also noted that some symptomatic EDS cases (with Parkinson diseases and the thalamic infarction) are not linked with hypocretin ligand deficiency.

Since CSF hypocretin measures are still experimental, cases with sleep abnormalities/cataplexy are habitually selected for CSF hypocretin measures. Therefore, it is still not known whether all or a large majority of cases with low CSF hypocretin-1 levels with CNS intervention exhibit EDS/cataplexy.

Occurrences of cataplexy in idiopathic narcolepsy cases are tightly associated with hypocretin ligand deficiency. However, this link is less clear in symptomatic cases. Since none of the acute and subacute symptomatic cases (such as MS, GBS, and ADEM) with undetectable CSF hypocretin-1 levels are found to develop cataplexy, chronic hypocretin deficiency may therefore be required to express cataplexy. Even when a very strict criterion for cataplexy is applied, about 10% of narcolepsy-cataplexy patients have normal CSF hypocretin-1 (2,4,7). Whether or not hypocretin neurotransmission is abnormal in these rare cases is unknown. Considering the fact that hypocretin production and hypocretin neurons appeared to be normal in hypocretin receptor 2-mutated narcoleptic Dobermans (131), it is possible that deficiencies in hypocretin receptors and a downstream pathway may exist in some of these patients. However, this cannot be tested currently. Similarly, it is not known whether narcoleptic subjects without cataplexy simply have milder neuropathology. Narcoleptic subjects without cataplexy may have sufficient hypocretin production to maintain normal CSF levels and stave off cataplexy, but the partial loss may still be great enough to produce sleepiness. With regard to this, it is also possible that some hypocretin non-deficient hypersomnia patients would show altered responses after various manipulations that normally increase hypocretin levels (i.e., exercise, sleep deprivation, food restrictions), if this was testable in humans.

A large number of HLA DR2/DQ6(DQB1*0602) negative symptomatic narcolepsy/EDS [53% (31/59) in narcolepsy and 87% (13/15) in EDS] were found [see Section IA and Ref. (40)]. The brain system critical for these sleep abnormalities (i.e., the hypocretin system) could be damaged by certain neurological conditions (such as tumors, vascular diseases). These cases are often associated with detectably-low or intermediate CSF hypocretin levels, and are in contrast to the undetectable idiopathic narcoleptic cases.

Nevertheless, increased HLA DR2/DQ6 (DQB1*0602) positively [47% (28/59)] was still observed in symptomatic narcoleptic cases. Although some HLA positive hypocretin-deficient symptomatic cases may be due to simple comorbilities of idiopathic narcolepsy, HLA may also play a role(s) in other cases: brain insult may trigger/facilitate the HLA-mediated hypocretin cell damage in which the mechanism may also be shared with that in the hypocretin deficient idiopathic cases of narcolepsy. Regarding hypocretin deficiency among immune-mediated neurological conditions, hypocretin deficiency with the hypothalamic lesions was noted in some MS and ADEM cases. In contrast, no clear local lesions were noted in hypocretin deficiency in GBS and Ma2 positive paraneoplastic syndromes. Thus, it appears that hypocretin ligand deficiency in GBS and Ma2 may possibly be more selective at the cellular level, and the mechanism involved in these conditions should be further studied.

Finally, further studies of the involvement of the hypocretin system in symptomatic narcolepsy and EDS are helpful to understand the pathophysiological mechanisms for occurrence of EDS and cataplexy. Measuring CSF hypocretin-1 may be also useful to choose treatment options, such as using wake-promoting compounds, anticataplectic medications and ultimately, for starting treatment with hypocretin agonists when they become available.

Acknowledgments

The authors wish to thank Ms. Chaya Y. for collecting many old papers and Dr. Szilagyi G. for translations from French or German to English.

References

1. Nishino S, Okura M, Mignot E. Narcolepsy: genetic predisposition and neuropharmacological mechanisms. Sleep Med Rev 2000; 4:57–99.
2. Mignot E, Lammers G, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
3. Nishino S, Ripley B, Overeem S, Lammers G, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
4. Nishino S, Ripley B, Overeem S, et al. Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. Ann Neurol 2001; 50:381–388.
5. Dalal M, Schuld A, Haack M, et al. Normal plasma levels of orexin A (hypocretin-1) in narcoleptic patients. Neurology 2001; 56:1749–1751.
6. Kanbayashi T, Inoue Y, Chiba S, et al. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002; 11:91–93.
7. Krahn L, Pankratz V, Oliver L, Boeve B, Silber M. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25: 733–736.
8. Bassetti C, Gugger M, Bischof M, et al. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.
9. Ebrahim I, Sharief M, de Lacy S, et al. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurosurg Psychiatry. 2003; 74:127–130.
10. de Lecea L, Kilduff T, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998; 95:322–327.
11. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.
12. Willie J, Chemelli R, Sinton C, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 2001; 24:429–458.
13. Ripley B, Overeem S, Fujiki N, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001; 57:2253–2258.
14. Kanbayashi T, Inoue Y, Kawanishi K, et al. CSF hypocretin measures in patients with obstructive sleep apnea. J Sleep Res 2003; 12:339–341.
15. Arii J, Kanbayashi T, Tanabe Y, et al. CSF hypocretin-1 (orexin-A) levels in childhood narcolepsy and neurologic disorders. Neurology 2004; in press.
16. von Economo C. Sleep as a problem of localization. J Nerv Ment Dis 1930; 71:249–259.
17. Adie J. Idiopathic narcolepsy: a disease sui generis, with remarks on the mechanism of sleep. Brain 1926; 49:257–306.
18. Kandt R, Emerson R, Singer H, Valle D, Moser H. Cataplexy in variant forms of Niemann-Pick disease. Ann Neurol 1982; 12:284–288.
19. Parkes J. Genetic factors in human sleep disorders with special reference to Norrie disease, Prader-Willi syndrome and Moebius syndrome. J Sleep Res 1999; 8:14–22.

20. Martinez-Rodriguez J, Lin L, Iranzo A, et al. Decreased hypocretin-1 (Orexin-A) levels in the cerebrospinal fluid of patients with myotonic dystrophy and excessive daytime sleepiness. Sleep 2003; 26:287–290.
21. Heyck H, Hess R. Zur Narkolepsiefrage, Klinik und Electroenzephalogramm. Fortschr. Neurol. Psychiat. 1954; 12:531–579.
22. Roth B. Narkolepsie a hypersomnies hlediska fysiologie spanku. Statni zkravotnicke nakladatelstvi. Prague, 1957.
23. Roth B. Narkolepsie und Hypersomnie vom Standpunkt der Physiologie des Schlafes. Berlin: VEB Verlag Volk und Gesundheit, 1962.
24. Devic M, Aimard P, Michel F, Masquin M. Clinical study of essential narcolepsies and cataplexies. Rev Neurol (Paris) 1967; 116:471–490.
25. Bonduelle M, Bouygues P, Delahousse J, Faveret C. Posttraumatic narcolepsy. Lille Med 1959; 4: 719–721.
26. Bonduelle M, Bouygues P, Delahousse J, Faveret C. Posttraumatic narcolepsy. Rev Otoneuroophtalmol 1959; 31:1–5.
27. Bonduelle M, Degos C. Symptomatic narcolepsies: a critical study. In: Guilleminault C, Dement W, Passouant P, eds. Narcolepsy. New York: Spectrum, 1976:313–332.
28. Autret A, Lucas B, Henry-Lebras F, de Toffol B. Symptomatic narcolepsies. Sleep 1994; 17:S21–S24.
29. Aldrich M, Naylor M. Narcolepsy associated with lesions of the diencephalon. Neurology 1989; 39:1505–1508.
30. Souques A. Narcolepsie d'origine traumatique: ses rapports avec une lesion de la region infundibulo-hypophysaire. Rev Neurol 1918; 33:521–524.
31. Francois H, Vernier L. Etude anatomo-clinique d'un cas tumeur de IIIeme ventricule cerebral. Rev Neurol 1919; 32:921–925.
32. Andre-Thomas A, Jumentie J, Chausseblanche. Lethargie intermittente traduisant l'existence d'une tumeur du IIIeme ventricule—observation anatomo-clinique. Rev Neurol (Paris) 1923; 39:67–73.
33. Alajouanine T, Lagrange, Baruk H. Tumeur de la glande pineale diagnostiquee cliniquement chez l'adulte. Bull Soc Med Hop Paris 1925; 49:1309–1314.
34. Lechelle P, Alajouanine T, Thevenard A. Deux cas de tumeur du lobe frontale a forme somnolente. Bull Soc Med Hop Paris 1925; 49:1347–1352.
35. Souques A, Baruk H, Bertrand I. Tumeurs de l'infudibulum avec lethargie isolee. Rev Neurol 1926; 1:532–540.
36. Lhermitte J, Tournay A. Le sommeil normal et pathologique. Rev Neurol 1927; 34:751-822, 885–887.
37. Lhermitte J, Kyriaco N. Hypersomnie periodique regulierement rythmee par les regles dans un cas de tumeur basilaire du cerveau. Rev Neurol 1929; 2:715–721.
38. Fulton J, Bailey P. Tumors in the third ventricle. J Nerv Ment Dis 1929; 69:1-25, 145-64, 261–277.
39. Lankford D, Wellman J, O'Hara C. Posttraumatic narcolepsy in mild to moderate closed head injury. Sleep 1994; 17:S25–S28.
40. Nishino S, Kanbayashi T. Symptomatic narcolepsy, cataplexy and hypersomnia, and their implications in the hypothalamic hypocretin/orexin system. Sleep Med Rev. 2005; 9:269–310.
41. Anderson M, Salmon M. Symptomatic cataplexy. J Neurol Neurosurg Psychiatry 1977; 40:186–191.
42. Stahl S, Layzer R, Aminoff M, Townsend J, Feldon S. Continuous cataplexy in a patient with a midbrain tumor: the limp man syndrome. Neurology 1980; 30:1115–1118.
43. Schoenhuber R, Angiari P, Peserico L. Narcolepsy symptomatic of a pontine glioma. Ital J Neurol Sci 1981; 2:379–380.
44. Pritchard P, Dreifuss F, Skinner R, Pickett J, Biggs P. Symptomatic narcolepsy. Neurology 1983; 33:239.
45. Schwartz W, Stakes J, Hobson J. Transient cataplexy after removal of a craniopharyngioma. Neurology 1984; 34:1372–1375.
46. Gurewitz R, Blum I, Lavie P, et al. Recurrent hypothermia, hypersomnolence, central sleep apnea, hypodipsia, hypernatremia, hypothyroidism, hyperprolactinemia and growth hormone deficiency in a boy—treatment with clomipramine. Acta Endocrinol Suppl (Copenh) 1986; 279.
47. Rubinstein I, Gray T, Moldofsky H, Hoffstein V. Neurosarcoidosis associated with hypersomnolence treated with corticosteroids and brain irradiation. Chest 1988; 94:205–206.
48. Kowatch R, Parmelee D, Morin C. Narcolepsy in a child following removal of a craniopharyngioma. Sleep Res. 1989; 18:250.
49. Onofrj M, Curatola L, Ferracci F, Fulgente T. Narcolepsy associated with primary temporal lobe B-cells lymphoma in a HLA DR2 negative subject. J Neurol Neurosurg Psychiatry. 1992; 55:852–853.

50. Clavelou P, Tournilhac M, Vidal C, Georget A, Picard L, Merienne L. Narcolepsy associated with arteriovenous malformation of the diencephalon. Sleep. 1995; 18:202–205.
51. Servan J, Marchand F, Garma L, Seilhean D, Hauw J, Delattre J. Narcolepsy disclosing neurosarcoidosis. Rev Neurol (Paris) 1995; 151:281–283.
52. Ma T, Ang L, Mamelak M, Kish S, Young B, Lewis A. Narcolepsy secondary to fourth ventricular subependymoma. Can J Neurol Sci. 1996; 23:59–62.
53. Malik S, Boeve B, Krahn L, Silber M. Narcolepsy associated with other central nervous system disorders. Neurology 2001; 57:539–541.
54. Marcus C, Trescher W, Halbower A, Lutz J. Secondary narcolepsy in children with brain tumors. Sleep 2002; 25:435–439.
55. Snow A, Gozal E, Malhotra A, et al. Severe hypersomnolence after pituitary/hypothalamic surgery in adolescents: clinical characteristics and potential mechanisms. Pediatrics. 2002; 110:e74.
56. Rosen G, Bendel A, Neglia J, Moertel C, Mahowald M. Sleep in children with neoplasms of the central nervous system: case review of 14 children. Pediatrics. 2003; 112:e46–e54.
57. Arii J, Kanbayashi T, Tanabe Y, Ono J, Nishino S, Kohno Y. A hypersomnolent girl with decreased CSF hypocretin level after removal of a hypothalamic tumor. Neurology 2001; 56:1775–1776.
58. Krahn L, Boeve B, Oliver L, Silber M. Hypocretin (orexin) and melatonin values in a narcoleptic-like sleep disorder after pinealectomy. Sleep Med 2002; 3:521–523.
59. Dempsey O, McGeoch P, de Silva R, Douglas N. Acquired narcolepsy in an acromegalic patient who underwent pituitary irradiation. Neurology 2003; 61:537–540.
60. Nokura K, Kanbayashi T, Ozeki T, et al. Hypersomnia Secondary To Hypothalamic Damage And CSF Orexin-A Level In Four Cases. Sleep 2004; 27:A249.
61. Gill A. Idiopathic and traumatic narcolepsy. Lancet. 1941:474–579.
62. Guilleminault C, Faull K, Miles L. Post-traumatic daytime sleepiness: a review of 20 patients. Neurology 1983; 33:1584–1589.
63. Maccario M, Ruggles K, Meriwether M. Post-traumatic narcolepsy. Mil Med 1987; 152:370–371.
64. Good J, Barry E, Fishman P. Posttraumatic narcolepsy: the complete syndrome with tissue typing. Case report. J Neurosurg 1989; 71:765–767.
65. Maeda M, Tamaoka A, Hayashi A, Mizusawa H, Shoji S. A case of HLA-DR2, DQw1 negative post-traumatic narcolepsy. Rinsho Shinkeigaku 1995; 35:811–813.
66. Francisco G, Ivanhoe C. Successful treatment of post-traumatic narcolepsy with methylphenidate: a case report. Am J Phys Med Rehabil 1996; 75:63–65.
67. Dauvilliers Y, Baumann C, Carlander B, et al. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and otherhypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry. 2003; 74:1667–1673.
68. Arii J, Kanbayashi T, Miyamoto H, Suzuki H, Kondo H, Ishii M. A case with post-trumatic hypersomnia and low hypocretin/orexin level. JSSR abstract 2004:257.
69. Bruck D, Broughton R. Diagnostic ambiguities in a case of post-traumatic narcolepsy with cataplexy. Brain Inj 2004; 18:321–326.
70. Berg O, Hanley J. Narcolepsy in two cases of multiple sclerosis. Acta Neurol Scand. 1963; 39:252–256.
71. Ekbom K. Familial multiple sclerosis associated with narcolepsy. Arch Neurol 1966; 15:337–344.
72. Schrader H, Gotlibsen O, Skomedal G. Multiple sclerosis and narcolepsy/cataplexy in a monozygotic twin. Neurology 1980; 30:105–108.
73. Younger D, Pedley T, Thorpy M. Multiple sclerosis and narcolepsy: possible similar genetic susceptibility. Neurology 1991; 41:447–448.
74. Iseki K, Mezaki T, Oka Y, et al. Hypersomnia in MS. Neurology 2002; 59:2006–2007.
75. Oka Y, Kanbayashi T, Mezaki T, et al. Low CSF hypocretin-1/orexin-A associated with hypersomnia secondary to hypothalamic lesion in a case of multiple sclerosis. J Neurol. 2004; 251:885–886.
76. von Economo C. Encephalitis lethargica: its sequela and treatment. London: Oxford University Press, 1931.
77. Stiefler G. Narkolepsie nach Enzephalitis Lethargica. Wien Klin 1924; 37:1044–1046.
78. Barker W. Studies in epilepsy: personality patterns, situatuonal stress and the symptoms of narcolepsy. Psychosom Med 1948; 10:193–202.
79. Smith C, Hamilton J. Psychological factors in the narcolepsy-cataplexy syndrome. Psychosom Med 1959; 21:40–49.
80. Mamelak M. A perspective on narcolepsy. Encephale 1992; 18:347–351.

81. Siegel J. Brainstem Mechanisms Generating REM Sleep. In: Kryger MR, T. Dement, WC., ed. Principles and Practice of Sleep Medicine. Philadelphia: W.B. Saunders Company, 2000:112–133.
82. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med. 2000; 6:991–997.
83. Thannickal T, Moore R, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
84. Melberg A, Ripley B, Lin L, Hetta J, Mignot E, Nishino S. Hypocretin deficiency in familial symptomatic narcolepsy. Ann Neurol 2001; 49:136–137.
85. Melberg A, Dahl N, Hetta J, et al. Neuroimaging study in autosomal dominant cerebellar ataxia, deafness, and narcolepsy. Neurology 1999; 53:2190–2192.
86. Scammell T, Nishino S, Mignot E, Saper C. Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke. Neurology. 2001; 56:1751–1753.
87. Vela-Bueno A, Kales A, Soldatos C, et al. Sleep in the Prader-Willi syndrome. Clinical and polygraphic findings. Arch Neurol. 1984; 41:294–296.
88. Helbing-Zwanenburg B, Kamphuisen H, Mourtazaev M. The origin of excessive daytime sleepiness in the Prader-Willi syndrome. J Intellect Disabil Res. 1993; 37:533–541.
89. Vgontzas A, Bixler E, Kales A, et al. Daytime sleepiness and REM abnormalities in Prader-Willi syndrome: evidence of generalized hypoarousal. Int J Neurosci. 1996; 87:127–139.
90. Manni R, Politini L, Nobili L, et al. Hypersomnia in the Prader Willi syndrome: clinical-electrophysiological features and underlying factors. Clin Neurophysiol 2001; 112:800–805.
91. Tobias E, Tolmie J, Stephenson J. Cataplexy in the Prader-Willi syndrome. Arch Dis Child 2002; 87:170.
92. Nevsimalova S, Vankova J, Stepanova I, Seemanova E, Mignot E, Nishino S. Hypocretin Deficiency in Prader-Willi syndrome. J Sleep Res 2004.
93. Vankova J, Stepanova I, Jech R, et al. Sleep disturbances and hypocretin deficiency in Niemann-Pick disease type C. Sleep 2003; 26:427–430.
94. Vanier M. Maladie de Niemann-Pick: etude biochimique de 107 cas (97 familles). Manifestations clinique et approche physiopathologique du type C. Lyon, 1983.
95. Kanbayashi T, Abe M, Fujimoto S, et al. Hypocretin deficiency in niemann-pick type C with cataplexy. Neuropediatrics 2003; 34:52–53.
96. Philip P, Ghorayeb I, Leger D, et al. Objective measurement of sleepiness in summer vacation long-distance drivers. Electroencephalogr Clin Neurophysiol 1997; 102:383–389.
97. Coccagna G, Mantovani M, Parchi C, Mironi F, Lugaresi E. Alveolar hypoventilation and hyperosmnia in myotonic dystrophy. J Neurol Neurosurg Psychiatry 1975; 38:977–984.
98. Park J, Radtke R. Hypersomnolence in myotonic dystrophy: demonstration of sleep onset REM sleep. J Neurol Neurosurg Psychiatry 1995; 58:512–513.
99. Gibbs Jr, Ciafaloni E, Radtke R. Excessive daytime somnolence and increased rapid eye movement pressure in myotonic dystrophy. Sleep 2002; 25:672–675.
100. Katz E, McGrath S, Marcus C. Late-onset central hypoventilation with hypothalamic dysfunction: a distinct clinical syndrome. Pediatr Pulmonol 2000; 29:62–68.
101. Marcus C, Mignot E. Letter to the editor regarding our previous publication: "Secondary narcolepsy in children with brain tumors," SLEEP 2002; 25:435–439. Sleep 2003; 26:228.
102. Kubota H, Kanbayashi T, Tanabe Y, et al. Decreased cerebrospinal fluid hypocretin-1 levels near the onset of narcolepsy in 2 prepubertal children. Sleep 2003; 26:555–557.
103. Tohyama J, Kanazawa O, Akasaka N, Kamimura T. A case of bilateral paramedian thalamic infarction in childhood with the sensory disturbance and the sensory loss of taste. No To Hattatsu 2004; 36:65–69.
104. Guilleminault C, Quera-Salva M, Goldberg M. Pseudo-hypersomnia and pre-sleep behaviour with bilateral paramedian thalamic lesions. Brain 1993; 116:1549–1563.
105. Bassetti C, Mathis J, Gugger M, Lovblad K, Hess C. Hypersomnia following paramedian thalamic stroke: a report of 12 patients. Ann Neurol 1996; 39:471–480.
106. Kashiwagi M, Tanabe T, Hara K, et al. Sleepiness Due To Wernicke's Encephalopathy Wirh Bilateral Hypothalamic Lesion In A 5-Year-Old Girl. Sleep 2004; suppl:A315.
107. Yamato H, Tanozaki M, Seino Y, Wakasaya Y, Suda T, Baba M. A hypersomnia case due to limbic encephalitis with decreased orexin level. Rinsho Shinkeigaku 2004; 44:482.
108. Lagrange A, Blaivas M, Gomez-Hassan D, Malow B. Rasmussen's syndrome and new-onset narcolepsy, cataplexy, and epilepsy in an adult. Epilepsy Behav 2003; 4:788–792.

109. Overeem S, van Hilten J, Ripley B, Mignot E, Nishino S, Lammers G. Normal hypocretin-1 levels in Parkinson's disease patients with excessive daytime sleepiness. Neurology 2002; 58:498–499.
110. Drouot X, Moutereau S, Nguyen J, et al. Low levels of ventricular CSF orexin/hypocretin in advanced PD. Neurology 2003; 61:540–543.
111. Baumann C, Dauvilliers Y, Mignot E, Bassetti C. Normal CSF Hypocretin-1 (Orexin A) Levels in Dementia with Lewy Bodies Associated with Excessive Daytime Sleepiness. Eur Neurol. 2004; 52:73–76.
112. Hattori Y, Hattori T, Mukai E, et al. Excessive daytime sleepiness and low CSF orexin-A/hypocretin-I levels in a patient with probable progressive supranuclear palsy. No To Shinkei 2003; 55:1053–1056.
113. Bliwise D, Rye D, Dihenia B, Gurecki P. Greater daytime sleepiness in subcortical stroke relative to Parkinson's disease and Alzheimer's disease. J Geriatr Psychiatry Neurol 2002; 15:61–67.
114. Billiard M. Other hypersomnias. In: Billiard M, ed. Sleep: Physiology, Investigations, and Medicine. New York: Kluwer Academic/Plenum Publishers, 2003.
115. Poirier G, Montplaisir J, Dumont M, et al. Clinical and sleep laboratory study of narcoleptic symptoms in multiple sclerosis. Neurology 1987; 37:693–695.
116. Kato T, Kanbayashi T, Yamamoto K, et al. Hypersomnia and low CSF hypocretin-1 (orexin-A) concentration in a patient with multiple sclerosis showing bilateral hypothalamic lesions. Intern Med 2003; 42:743–745.
117. Kubota H, Kanbayashi T, Tanabe Y, Takanashi J, Kohno Y. A case of acute disseminated encephalomyelitis presenting hypersomnia with decreased hypocretin level in cerebrospinal fluid. J Child Neurol 2002; 17:537–539.
118. Gledhill R, Bartel P, Yoshida Y, Nishino S, Scammell T. Narcolepsy caused by acute disseminated encephalomyelitis. Arch Neurol. 2004; 61:758–760.
119. Yoshikawa S, Suzuki S, Yamamoto K, et al. A case of acute disseminated encephalomyelitis associated with hypersomnia and low CSF hypocretin levels. Pediatric Neurology 2004.
120. Yano T, Kanbayashi T, Sawaishi Y, Shimizu T, Takada G. An Infant Case Of Hypersomnia With Acute Disseminated Encephalomyelitis Due To Hypothalamic Dysfunction. Sleep 2004; 27:A238.
121. Nozaki H, Katada S, Sato M, Tanaka K, Nishizawa M. A case with hypersomnia and paresthesia due to diffuse MS leisons from hypothalamus to spine. Rinsho Shinkeigaku 2004; 44:59.
122. Mignot E, Nishino S. Perspectives for new treatments: a 2005 update. Sleep 2005; 28.
123. Yamashita S, Ueno K, Hashimoto Y, Teramoto H, Uchino M. A case of acute disseminated encephalomyelitis accompanying Mycoplasma pneumoniae infection. No To Shinkei 1999; 51:799–803.
124. Kanbayashi T, Goto A, Hishikawa Y, et al. Hypersomnia due to acute disseminated encephalomyelitis in a 5-year-old girl. Sleep Med 2001; 2:347–350.
125. Kanbayashi T, Ishiguro H, Aizawa R, et al. Hypocretin-1 (orexin-A) concentrations in cerebrospinal fluid are low in patients with Guillain-Barre syndrome. Psychiatry Clin Neurosci. 2002; 56:273–274.
126. Nishino S, Kanbayashi T, Fujiki N, et al. CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2003; 61:823–825.
127. Baumann C, Bassetti C. CSF hypocretin levels in Guillain-Barre syndrome and other inflammatory neuropathies. Neurology 2004; 62:2337.
128. Overeem S, Dalmau J, Bataller L, et al. Hypocretin-1 CSF levels in anti-Ma2 associated encephalitis. Neurology. 2004; 62:138–140.
129. Lecendreux M, Maret S, Bassetti C, Mouren M, Tafti M. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old boy. J Sleep Res 2003; 12:347–348.
130. Mignot E. Sleep, sleep disorders and hypocretin (orexin). Sleep Med 2004; 5:S2–S28. Ripley B, Fujiki N, Okura M, Mignot E, Nishino S. Hypocretin levels in sporadic and familial cases of canine narcolepsy. Neurobiol Dis 2001; 8:525–534.
131. Castillo PR, Mignot E, Woodruff BK, Boeve BF. Undetectable CSF hypocretin-1 in "Hashimoto's encephalopathy" associated with coma. Neurology 2004; 62(10):1909.
132. Voderholzer U, Riemann D, Gann H, et al. Transient total sleep loss in cerebral Whipple's disease: a longitudinal study. J Sleep Res 2002; 11(4):321–329.

30
Poststroke and Posttraumatic Hypersomnia

CLAUDIO L. BASSETTI and CHRISTIAN R. BAUMANN
University Hospital, Zurich, Switzerland

I. Introduction

The first theories about the existence of specific sleep and wakefulness centers in the brain were based upon observations of hypersomnia and insomnia in patients with disorders of the central nervous system. Trömner, for example, believed in the existence of a sleep center in the thalamus (1). On the other hand, Von Economo—on the base of his observations of sleep-wake disturbances following encephalitis lethargica—postulated the existence of a wakefulness-promoting area in the posterior hypothalamus and of a sleep-promoting area in the anterior thalamus (2).

Although hypersomnia following stroke was mentioned by MacNish already in 1830 subsequent reports on the occurrence of sleep-wake disorders in patients with cerebrovascular diseases remained scarce until the beginning of the 20th century (3,4). Lhermitte and Tournay were among the firsts to report posttraumatic hypersomnia (5).

II. Epidemiology

The incidence of stroke is about 300–600/100,000/year (6). The frequency of hypersomnia after stroke is essentially unknown but may be as high as 20–40% (7). In a consecutive series of 100 patients with ischemic stroke, 22 patients reported at one month after stroke excessive daytime sleepiness and/or an increase in sleep needs as compared to the prestroke situation (8). Patients with and without poststroke hypersomnia were similar in age (52 ± 13 vs. 55 ± 12), gender, Epworth sleepiness score (6 ± 5 vs. 5 ± 4) and estimated sleep needs in hours/d (7 ± 1 vs. 7 ± 1) *before* stroke, topography of stroke (thalamic/brainstem in 9% vs. 31%) and apnea-hypopnea-index (16 ± 13 vs. 16 ± 14) was assessed within the first 1–2 weeks *after* stroke. Conversely, patients with poststroke hypersomnia had a more severe stroke (NIH-stroke scale 12 ± 6 vs. 6 ± 3, $p < 0.0001$) and worse short-term outcome (Rankin score 3.0 ± 0.15 vs. 1.7 ± 1.2, $p = 0.001$). The less well defined symptom "fatigue" may be found in an even higher percentage of poststroke patients (9).

The incidence of traumatic brain injury (TBI) is about 60–300 per 100,000/year (10). The frequency of hypersomnia after TBI is poorly unknown. In a retrospective analysis of 71 patients, 38 months after TBI 21% of patients had an Epworth Sleepiness

Scale (ESS) score >10, and 47% of patients had a mean sleep latency ≤10 minutes on multiple sleep latency tests (MSLT) (11). In a prospective study of 75 patients, six months after TBI excessive daytime sleepiness (ESS > 10) was reported by 30% of patients and an increase in sleep needs (≥2 hours sleep/day as compared to the pre-TBI situation) was reported by 19% of patients (Baumann et al., in preparation). A mean sleep latency <10 minutes on MSLT was found in 57% (<5 minutes in 21%) of patients.

III. Clinical Features

Hypersomnia can be defined clinically as a reduced latency to sleep, increased sleep needs/behavior (hypersomnia "sensu strictu"), excessive daytime sleepiness (with or without increase napping and "sleep attacks"), or a combination of these symptoms. After stroke and TBI a continuum (or co-occurence) exists between hypersomnia, fatigue and depression. This continuum is illustrated in single patients by the evolution from initial coma and subsequent severe hypersomnia to mild hypersomnia, apathy and depression in the recovery phase.

A. Poststroke Hypersomnia

The semiological spectrum of poststroke hypersomnia is wide and varies according to stroke topography (7,8,12).

In deep (subcortical) hemispheric and particularly paramedian thalamic lesions hypersomnia may correspond to a so-called presleep behavior, during which patients yawn, stretch, close their eyes, curl up and assume a normal sleeping posture, while complaining of a constant sleep urge (13). Some of these patients are able to control this behavior when stimulated or given explicit, active tasks to perform. This behavior may be compulsive in that removal of the patient from bed can result in repeated attempts to lie down and adopt a sleeping posture. For this dissociation between lack of autoactivation in the presence of preserved heteroactivation, first described in post-encephalitic parkinsonism by Naville in 1922, the terms bradyphrenia, athymormia, "pure psychic akinesia" and "instruction dependant behavior" were suggested (14).

In some patients with deep frontal, thalamic or midbrain lesions, hypersomnia evolves to extreme apathy with lack of spontaneity and initiative, slowness, poverty of movement and catalepsy, a condition for which the term akinetic mutism was coined.

Fatigue, defined as a feeling of physical and/or mental tiredness and lack of energy, is often characterized also by a strong desire for sleep, despite an often normal or (paradoxically) decreased sleep propensity. The high frequency of fatigue, which affects up to 68% of patients 3 to 13 months after stroke, has only recently been recognized (15). Fatigue does not seem to be linked to time poststroke, stroke severity, or lesion location. A significant overlap exists particularly between poststroke fatigue and poststroke depression. However, poststroke fatigue may occur in the absence of depression and even persist after recovery from the neurological deficits (16).

Hypersomnia with hyperphagia (Kleine-Levin–like syndrome) was reported after multiple cerebral strokes (17).

Narcolepsy-like syndromes (symptomatic narcolepsy) have been described overall in six patients in the literature after thalamic, subthalamic (hypothalamic)

and pontine stroke (18–20). The patient with subthalamic stroke had no cataplexy but low CSF hypocretin-1 levels (19).

A continuum exists between hypersomnia, athymormia, akinetic mutism and fatigue. In some patients, episodes of hypersomnia, mutism and akinesia alternate with episodes of insomnia, psychomotor agitation or confusion state (7). Façon and co-workers described, for example, a 78-year-old patient with bilateral tegmental thalamo-mesencephalic stroke presenting with bilateral nerve III palsy, hallucinations, inversion of sleep-wake cycle and severe hypersomnia persisting until death three years later (21). We have observed a 68-year-old patient with a left subcortical infarct and mild neurological deficits (NIH stroke scale = 6) in whom a profound inversion of sleep-wake cycle was first noted. Two weeks later daytime hypersomnia had almost recovered while nighttime insomnia with estimated 2–3 hours of sleep per night was still present. Sleep-wake functions finally normalized four weeks after stroke onset (7).

B. Posttraumatic Hypersomnia

After TBI a variety of sleep-wake disorders including hypersomnia, excessive daytime sleepiness (EDS), narcolepsy, insomnia, sleep-disordered breathing, and delayed sleep phase syndrome have been reported (11,22–25). Unfortunately, most series are either retrospective or based on small series with variable intervals after TBI.

In a study of 20 patients with EDS after TBI, Guilleminault et al. found abnormal MSLT findings in 18 patients (90%). Eight of these patients had sleep apnea. One other patient reported cataplexy-like episodes and had 2 SOREM on MSLT (22). In a second study in 184 patients with EDS after head-neck trauma, the same group reported hypnagogic hallucinations in 53% of the patients, sleep paralysis in 15% and REM sleep behavior disorder in 9%. Patients with severe trauma (coma >24 hours, head fracture, or immediate neurosurgical interventions) were more likely to have ESS scores >16 and mean sleep latencies on MSLT <5. Sleep-apnea was commonly found in these patients (32%) (23).

Parcell et al. reported subjective EDS (defined by an ESS score >9) in 19% of 63 consecutive TBI patients recruited after discharge from rehabilitation (mean time postinjury of 230 days, range 20–1194 days) (25). Masel et al. documented objective EDS (defined by a mean sleep latency on MSLT $<$ 10 minutes) in 47% of 71 TBI patients (mean time postinjury 38 $\pm$ 60 months) (26).

Narcolepsy-like syndromes (symptomatic narcolepsy) following TBI have been reported in 19 patients in the literature (20), of whom 14 had both EDS and cataplexy (see also for more details in this book the chapters on "symptomatic narcolepsy" and on the "spectrum of narcolepsy"). Although a few cases following severe head trauma are known, most patients reported suffered of a mild to moderate TBI and no correlation can be found between lesion topography and the appearance of posttraumatic narcolepsy (20). Considering also the HLA-positivity (DQB1*0602) in more than 30% of these patients (20), the variable interval between trauma and onset of sleep-wake symptoms [17–30 months in one series (27)], and the almost invariable absence of data on CSF hypocretin-1 levels in this population [intermediate levels in two patients and normal levels in a third one, (28–30)] the differentiation between "secondary/symptomatic" narcolepsy and "primary/idiopathic" narcolepsy triggered by TBI (and or acute stress) remains often difficult.

A few patients were reported to develop isolated cataplexy-like episodes that is without hypersomnia) following TBI, although the characteristics of these episodes raise some doubts about the exact nature of these spells (31,32).

C. Recovery of Poststroke and Posttraumatic Hypersomnia

The pattern of recovery of hypersomnia after TBI or stroke is highly variable. Extension and side of the lesions but also age and, possibly, genetic factors influence the recovery process. In most cases hypersomnia improves within a few days to weeks. In fact, hypersomnia within the first weeks after brain damage does not seem to preclude a good long-term outcome. Conversely, persistent hypersomnia over weeks or months often heralds long-term disability. In the most severely affected patients significant hypersomnia can persist over years. In other patients hypersomnia evolves to akinetic mutism with normal wakefulness but apathy, amnesia, attentional deficits, diminished psychomotor drive, and depressed mood. Finally, in less severe cases, patients may report at follow-up only a mildly prolonged nighttime sleep and daytime napping. Associated disturbances of attention and short-term memory, indifference, lack of initiative and mood flattening with depression may dominate the clinical picture (33).

IV. Pathophysiology

In patients with brain damage, sleep-wake disturbances (SWD) including hypersomnia can be of multifactorial origin. In addition to brain damage *per se*, environmental factors including noise, light, and intensive medical monitoring may contribute to the development of SWD. In fact, several studies have suggested that milder TBI are associated with greater frequency of SWD (34). Furthermore, sleep-disordered breathing, seizures, infections, fever, and drugs may aggravate sleep fragmentation and result in sleep disturbances. Anxiety, depression and psychological distress (difficulties in coping with brain damage in general) are frequently present and contribute to SWD (35).

Considering the complexity in anatomy and physiology of sleep-wake control, focal brain damage in the course of stroke and TBI are expected, however, *per se* to impair wakefulness, sleep or both in different ways according to extension, and topography of the lesion.

The following considerations are focussed on mechanisms involved in hypersomnia as direct consequence of brain damage. In general, hypersomnia following stroke allows more precise neuroanatomical and physiological considerations then hypersomnia after TBI, in which the frequent presence of multiple lesions and/or diffuse shearing injury preclude a direct correlation between sleep-wake symptoms and topography of the underlying lesion(s).

A. Neuroanatomical Considerations

The most severe and persistent forms of hypersomnia are observed in association with lesions in the paramedian thalamus, midbrain or upper pons. The paramedian thalamic stroke syndrome represents the most striking form of hypersomnia following focal brain damage. Patients usally present with a clinical (“diencephalic”) triade consisting

of initial coma/severe hypersomnia, vertical gaze palsy and neuropsychological deficits including amnesia (36–39). The lesion is typically centered in the dorsomedial and centromedian nuclei of the thalamus with variable extension to the anterior thalamus and the posterior hypothalamic/upper midbrain tegmentum. Hypersomnia can be seen also with unilateral lesions but rarely persists, in these cases, for more than a few months.

Less commonly hypersomnia may complicate lesions in the caudate, striatum, lower pons, medial medulla, and cerebral hemispheres (with or without mass effect) (7,36). The degree and duration of hypersomnia over time in these patients are less severe than in patients with paramedian thalamic stroke.

Brain lesions initially may cause coma or, conversely, manic delirium, hyperalertness, and insomnia before hypersomnia evolves. These observations point to a dual role of most brain regions involved in the regulation of the sleep-wake cycle, as originally suggested already by animal stimulation experiments (40).

B. Neurophysiological Considerations

Hypersomnia following focal brain damage is usually considered to reflect a decreased arousal secondary to a dysfunction of the wakefulness maintaining neuronal systems (ascending reticular activating system, ARAS). The term of "*passive" hypersomnia* was suggested by Passouant for hypersomnia resulting from such a "de-arousal" (41). The most severe and persisting forms of "de-arousal" are seen in patients with bilateral paramedian thalamic and brainstem lesions, where fibers of the ARAS are bundled and can be severely injured even by single small lesions. In large hemispheric strokes, de-arousal results from disruption of the ARAS in the upper brainstem secondary to vertical (transtentorial) or horizontal displacement of the brain due to brain edema. The occurrence of hypersomnia following cortical or striatal (e.g., caudate) strokes without mass effect (7), supports the assumption of a role of these structures in maintenance of arousal and more generally in sleep-wake regulation (42,43).

The possibility of a deficient sleep consolidation with a consequent sleep prolongation reflecting a reduced spindle activity has been discussed as a pathophysiological component of hypersomnia in supratentorial and particularly thalamic lesions (37,44).

Hypersomnia with increased sleep per 24 hours ("*active hypersomnia*," "hypersomnia sensu strictu") has rarely been documented in patients with thalamic, mesencephalic and pontine stroke (7,8). The most striking example of increased sleep is seen in patients with bilateral paramedian thalamic stroke. Arpa et al. reported a 44 year-old man with a right lateral-tegmental pontine hematoma and severe hypersomnia, in whom long-term EEG monitoring showed increased amounts of sleep ranging from 11 to 15 hours per day during the first 3 months after his stroke event. The relative amounts of slow wave sleep (4–11% of total sleep time) and REM sleep (8–10%) were slightly above normal values (45). Bastuji et al. described a patient with severe hypersomnia due to bilateral thalamo-mesencephalic stroke with an initial sleep behavior over 18 hours per day. Eight months after stroke hypersomnia had regressed clinically to 12 hours per day. By EEG criteria sleep was similarly present over about 12 hours per day, with an increase in both slow wave sleep (30% of total sleep time) and REM sleep (22%) (46). Popoviciu et al. presented a patient with akinetic

mutism due to bilateral, ventro-tegmental pontine stroke in whom a polygraphic recording demonstrated an increase of REM sleep (317 min/24 hours) and wakefulness (541 min/24 hours) (47).

C. Neurochemical Considerations

In a 23-year-old patient with bilateral diencephalic stroke following surgical removal of a craniopharyngeoma, CSF hypocretin-1 levels were intermediate (167 pg/ml), suggesting a link between poststroke hypersomnia and a deficient hypocretin neurotransmission (19). This HLA-negative patient reported also cataplexy-like episodes (weakenss while laughing of the neck and right arm muscles) and on MSLT had a mean sleep latency of 0.5 min and SOREM. It is noteworthy that the postoperative course in this patient was complicated by meningitis and hydrocephalus and that the clinical picture also included panhypopituitarism and obesity.

In three own patients with hypersomnia following paramedian thalamic (n = 2, Fig. 1) and pontine stroke (n = 1, Fig. 2), respectively, we found however normal CSF hypocretin-1 levels [two of these patients have been reported before, (28)]. Nishino and Kanbayashi recently reported a 15-year old male with hypersomnia

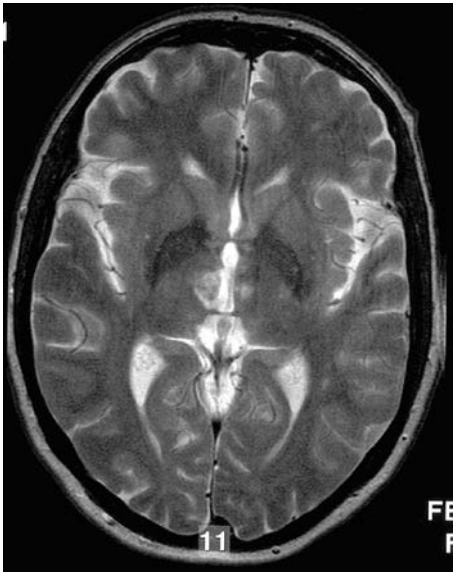


Figure 1 54-year-old patient with bilateral paramedian thalamic stroke. Poststroke vertical gaze palsy, severe amnesia, childish behavior, dysarthrophonia, increase in sleep needs (12 hr/day vs. 7 hr/day before stroke), mild excessive daytime sleepiness (EDS), loss of dream recall, and weight gain. Levels of hypocretin-1 in the cerebrospinal fluid are normal. Improvement of EDS/hypersomnia is achieved with levodopa 250 mg/d (modafinil is discontinued because of headaches). Fourteen months after stroke sleep needs have improved (10 hr/day) and dreaming has reappeared whereas memory deficits persist. *Source*: Courtesy of Prof. A. Valavanis, Institute of Neuroradiology, University Hospital, Zurich, Switzerland.

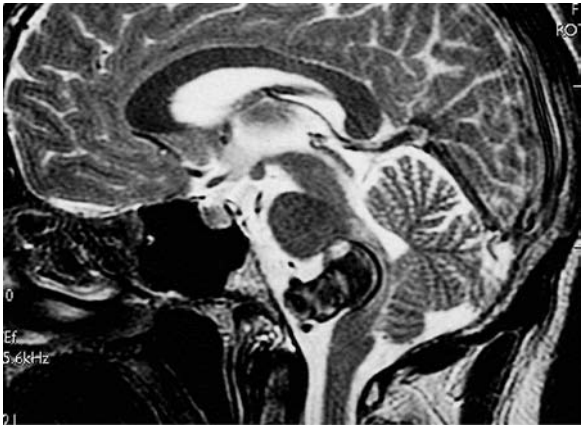


Figure 2 40-year-old patient with ponto-medullary stroke following embolization of a giant aneurysm of the basilar artery. Poststroke severe excessive daytime sleepiness (EDS, Epworth sleepiness score: 23/24) and increased sleep needs (12–14 hr/day). Polysomnography: sleep efficiency 97%, slow wave sleep 8%, no sleep apnea, no periodic limb movements in sleep. Multiple sleep latency test: mean sleep latency one minute, no sleep onset REM periods. Actigraphy: time "asleep" (rest or sleep) during 43% of the recording time (2 weeks). Normal cerebrospinal fluid levels of hypocretin-1. Patient does not wish any treatment for his EDS/hypersomnia. *Source*: Courtesy of Prof. A. Valavanis, Institute of Neuroradiology, University Hospital, Zurich, Switzerland. CSF hypocretin-1 data from Ref. 28; from Ref. 7.

following bilateral paramedian thalamic stroke, in whom CSF hypocretin-1 levels were also within normal limits (20).

In a series of 44 consecutive patients with acute TBI low/undetectable levels of (mostly ventricular) CSF hypocretin-1 were found in 84% of patients (48). Low/undetectable levels were associated with severe TBI as assessed by Glasgow Coma Scale (GCS) (GCS <12) and with pathological CT findings of the brain. This systematic study confirms observations of low hypocretin levels in single TBI patients, in whom clinical features and interval between TBI and CSF assessments were, however, not specified (49). More studies are needed to determine (*i*) the evolution of CSF hypocretin-1 levels over time; (*ii*) the potential link between deficient hypocretin neurotransmission and long-term outcome (including presence of hypersomnia) in patients with TBI.

The beneficial effect of levodopa or dopamine agonists in a few patients with hypersomnia following stroke or TBI (see below) suggests a role of this neurotransmitter system in the pathophysiology of these disorders.

V. Diagnosis

The recognition of posttraumatic and -stroke hypersomnia occurs primarily on clinical grounds. A full neurological, neuropsychiatric and polysomnographic work-up is

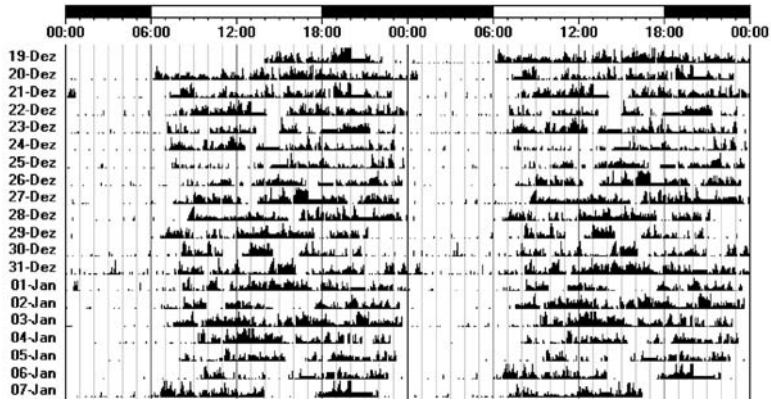


Figure 3 Actigraphy post-brain damage.

usually needed to rule out the presence of a "secondary" hypersomnia due to sleep disordered breathing, depression, medications, and so on.

In patients with "primary" hypersomnia (as a consequence of brain damage "*per se*") the correlation between clinical symptoms and sleep EEG findings is often poor (50). Sleep EEG may, for example, reveal both a reduction, less commonly an increase, of NREM and/or REM sleep (7,8). In hypersomnia following paramedian thalamic infarcts, sleep-like behavior may be accompanied by a variety of EEG patterns including diffuse low-voltage alpha-beta activity, NREM stage 1 sleep, slow wave activity, and REM sleep (37,38). For the same reasons, the MSLT may be also be inadequate for assessment of posttraumatic and -stroke hypersomnia, particularly in patients with thalmic or extensive cortico-subcortical lesions (37). This potential discrepancy between clinical symptomatology and sleep EEG findings can explain the use of different, in parts contradictory terms in the literature to describe similar clinical syndromes. For example, the terms hypersomnia, pseudohypersomnia, NREM 1-hypersomnia, subwakefulness syndrome, insomnia, and "agrypnia agitata" have been used by different groups to describe patients with similar sleep-wake disturbances following thalamic and cortico-subthalamic lesions (51).

Actigraphy may be helpful to estimate changes in sleep-wake rhythms and sleep/rest needs following brain damage as well as their evolution over time (Fig. 3) (8).

VI. Treatment

A link between severity of sleep-wake disturbances/sleep EEG changes and outcome of stroke and TBI has been reported (50,52–56). Diagnosis (and treatment) of posttraumatic and -stroke sleep-wake disorders may therefore have an impact on functional and cognitive outcome after brain damage. Treatment of postlesional hypersomnia is often ineffective. In single patients some improvement was seen in thalamic and mesencephalic lesions with amphetamines, modafinil, methylphenidate and dopaminergic agents. In patients with paramedian thalamic stroke treatment with 20–40 mg of

bromocriptine may improve apathy and presleep behavior. Improvement of alertness with 200 mg of modafinil has been reported in patients with bilateral mesodiencephalic paramedian infarct. Treatment of an associated depression with stimulating antidepressants may also improve postlesional hypersomnia. It is noteworthy that a favorable influence on early poststroke rehabilitation was reported for both methylphenidate (5–30 mg/d, 3 weeks-trial) and levodopa (100 mg/d, 3 weeks-trial), an effect that may at least in parts be related to improved alertness in these patients (57,58). One off-label use study reported a successful use of modafinil in 10 patients with excessive daytime sleepiness after TBI. Wakefulness and feeling of normality increased within 1–2 weeks of taking the drug (59). Objective measures of improvement, however, were not reported. In posttraumatic delayed sleep phase syndrome, chrono- and phototherapy have been used with success, whereas pharmacologic agents such as sedative/hypnotics, vitamins and melatonin have been only rarely of help (60).

VII. Conclusions

Despite a high frequency of hypersomnia in patients surviving stroke and TBI and the potential link between sleep-wake disturbances and functional outcome only few systematic studies have addressed this problem. Lesions in paramedian thalamus, midbrain and upper pons are usually responsible for the most severe and persistent forms of hypersomnia. Hypersomnia is thought to arise from a dysfunction of the arousal system and possibly also from an insufficient capacity to consolidate sleep, whereas a "true" excess of sleep represents a rare finding. The possibility of secondary forms of hypersomnia (e.g., due to sleep disordered breathing) and of a poor correlation between sleep-wake complaints and sleep EEG should be considered in the diagnostic work up, which may include actigraphy. Treatment with levodopa, dopamine agonists, stimulants (e.g., modafinil) and antidepressants may be of help in some patients. A better understanding of hypersomnia following TBI and stroke may improve the faith of large groups of patients also give new insights into the brain mechanisms responsible for sleep-wake functions.

References

1. Trömner E. Funktion und Lokalisation des Schlafes. Archive für Psychiatrie 1929; 86:284–287.
2. Von Economo C. Encephalitis lethargica. Wiener Medizinische Wochenschrift 1923; 73:777–782, 835–838, 1113–1118, 243–249, 334–338.
3. Claude H, Loyez M. Ramollissement du noyau rouge. Revue Neurologique 1912; 23:40–51.
4. Freund SC. Zur Klinik und Anatomie der vertikalen Blicklähmung. Neurologisches Zentralblatt 1913; 32:1215–1229.
5. Lhermitte J, Tournay A. Rapport sur le sommeil normal et pathologique. Revue Neurologique 1927:751–823.
6. Sudlow CL, Warlow CP. Comparable studies of the incidence of stroke and its pathological types: Results from an international collaboration. International Stroke Incidence Collaboration. Stroke 1997; 28(3):491–499.
7. Bassetti C. Sleep and Stroke. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. 4th ed. Philadelphia: Elsevier Saunders, 2005:811–830.
8. Bassetti CL, Valko P. Poststroke hypersomnia. Sleep Medicine Clinics 2006; 1(1):139–155.

9. Carlsson GE, Moller A, Blomstrand C. Consequences of mild stroke in persons <75 years- a 1 year follow-up. Cerebrovasc Dis 2003; 16(4):383–388.
10. Basso A, Previgliano I, Duarte JM, Ferrari N. Advances in management of neurosurgical trauma in different continents. World J Sureg 2001; 25(9):1174–1178.
11. Masel BE, Scheibel RS, Kimbark T, Kuma ST. Excessive daytime sleepiness in adults with brain inuries. Arch Phys Med Rehab 2001; 82(11):1526–1532.
12. Bassetti CL. Sleep and stroke. Seminars in Neurology 2005; 25(1):19–32.
13. Catsman-Berrevoets CE, Harskamp F. Compulsive pre-sleep behaviour and apathy due to bilateral thalamic stroke. Neurology 1988; 38:647–649.
14. Laplane D, Baulac M, Widlöcher D, Dubois B. Pure psychic akinesia with bilateral lesions of basal ganglia. Journal of Neurology, Neurosurgery, and Psychiatry 1984; 47:377–385.
15. Ingles JL, Eskes GA, Philipps SJ. Fatigue after stroke. Arch Phys Med Rehab 1999; 80(2):173–178.
16. Choi-Kwon S, Han SW, Kwon SI, et al. Poststroke fatigue: characteritics and related factors. Cerebrovasc Dis 2005; 19:84–90.
17. Drake ME. Kleine-Levine syndrome after multiple cerebral infarctions. Psychosomatics 1987; 28(6):329–330.
18. Rivera VM, Meyer JS, Hata T, Ishikawa Y, Imai A. Narcolepsy following cerebral hypoxic ischemia. Annals of Neurology 1986; 19:505–508.
19. Scammell TE, Nishino S, Mignot E, Saper CB. Narcolepsy and low CSF orexin (hypocretin) concentration after stroke. Neurology 2001; 56:1751–1753.
20. Nishino S, Kanbayashi T. Symptomatic narcolepsy, cataplexy, and hypersomnia, and their implications in the hypothalamic hypocretin/orexin system. Sleep Med Rev 2005; 9:269–310.
21. Façon E, Steriade M, Wertheim N. Hypersomnie prolongée engendrée par des lésions bilatérale du système activateur médial. Le syndrome thrombotique de la bifurcation du tronc basilaire. Revue Neurologique 1958; 98:117–133.
22. Guilleminault C, Faull KF, Miles L, van den Hoed J. Posttraumatic excessive daytime sleepiness: A review of 20 patients. Neurology 1983; 33:1584–1589.
23. Guilleminault C, Yuen KM, Gulevich MG, Karadeniz D, Leger D, Philip P. Hypersomnia after head-neck trauma: A medicolegal dilemma. Neurology 2000; 54(3):653–659.
24. Quinto C, Gllido C, Chokroverty S, Masdeu J. Posttraumatic delayed sleep phase syndrome. Neurology 2000; 54(1):250–252.
25. Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR. Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehab 2006; 87:278–285.
26. Masel BE, Scheibel RS, Kimbark T, Kuna ST. Excessive daytime sleepiness in adults with brain injuries. Arch Phys Med Rehab 2001; 82:1526–1532.
27. Lankford DA, Wellmann JJ, O'Hara C. Posttraumatic narcolepsy in mild to moderate closed head injury. Sleep 1994; 17:25–28.
28. Bassetti C, Gugger M, Bischof M, et al. The narcoleptic borderland: A multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.
29. Dauvilliers Y, Baumann CR, Maly FE, Billiard M, Bassetti C. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, other hypersomnias and neurological conditions. Journal of Neurology, Neurosurgery and Psychiatry 2003; 74(1667–1673).
30. Arii J, Kanbayashi T, Tanabe Y, et al. Hypersomnolent girl with decreased CSF hypocretin level after removal of a hypothalamic tumor. Neurology 2001; 56:1775–1776.
31. Ethelberg S. Symptomatic "cataplexy" or chalastic fits in cortical lesions of the frontal lobe. Brain 1950; 73:499–512.
32. Servan J, Marchans F, Garma L, Rancurel G. A case of non-narcoleptic post-traumatic cataplexy. Neurophysiol Clin 1996; 26(2):115–116.
33. Hermann DM, Mathis J, Wachter K, Achermann P, Bassetti C. Neurological, neuropsychological and sleep-wake disturbances in paramedian thalamic stroke: A follow-up study of 42 patients. (in preparation) 2006.
34. Mahmood O, Rapport LJ, Hanks RA, Fichtenberg NL. Neuropsychological performance and sleep disturbance follwoing trajmatic brain injury. J Head Trauma Rehabil 2004; 19:378–390.
35. Ruff RL. Two decades of advances in understanding of mild traumatic brain injury. J Head Trauma Rehabil 2005; 20:5–18.

36. Castaigne P, Escourolle R. Etude topographique des lésions anatomiques dans les hypersomnies. Revue Neurologique 1967; 116:547–584.
37. Bassetti C, Mathis J, Gugger M, Lövblad K, Hess CW. Hypersomnia following thalamic stroke. Ann Neurol 1996; 39(4):471–480.
38. Guilleminault C, Quera-Salva MA, Goldberg MP. Pseudo-hypersomnia and pre- sleep behaviour with bilateral paramedian thalamic lesions. Brain 1993; 116:1549–1563.
39. Castaigne P, Lhermitte F, Buge A, Escourelle R, Hauw JJ, Lyon-Caen O. Paramedian thalamic and midbrain infarcts: Clinical and neuropathological study. Annals of Neurology 1981; 10:127–148.
40. Hösli L. Dämpfende und fördernde Systeme im medialen Thalamus und im Retikularapparat (Nachweis der funktionellen Dualität durch selektive Reizungen und Ausschaltungen). Inauguraldissertation. Basel, Buchdruckerei Birkhäuser AG 1962.
41. Passouant P, Cadilhac J, Baldy-Moulinier M. Physio-pathologie des hypersomnies. Revue Neurologique 1967; 116:585–629.
42. Villablanca JR, Marcus RJ, Olmstead CE. Effect of caudate nuclei or frontal cortex ablations in cats. II. Sleep-wakefulness, EEG, and motor activity. Exp Neurol 1976; 53:31–50.
43. Braun AR, Balkin TJ, Wesensten NJ, et al. Regional cerebral blood flow throughout the sleep-wake cycle. An H215O study. Brain 1997; 120:1173–1197.
44. Gottselig J, Bassetti C, Achermann P. Power and coherence of sleep spindle activity following hemispheric stroke. Brain 2002; 125:373–385.
45. Arpa J, Rodriguez-Albarino R, Izal E, Sarria J, Barreiro ML. Hypersomnia after tegmental pontine hematoma: Case report. Neurologia (Spain) 1995; 10(3):140–144.
46. Bastuji H, Nighoghossian N, Salord F, Trouillas P, Fischer C. Mesodiencephalic infarct with hypersomnia: sleep recording in two cases. Journal of Sleep Research 1994; 3(suppl1):16.
47. Popoviciu L, Asgian B, Corfarici D, et al. Anatomoclinical and polygraphic features in cerebrovascular diseases with disturbances of vigilance. In: Tirgu-Mures L, Popoviciu L, Asgia B, et al., eds. Sleep 1978: Fourth European Congress on Sleep Research. New York: S. Karger, 1980:165–169.
48. Baumann CR, Stocker R, Imhof HG, et al. Hypocretin-1 (orexin A) deficiency in acute traumatic brain injury. Neurology 2005; 65:147–149.
49. Ripley B, Overeem N, Fujiki N, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001; 57:2253–2258.
50. Vock J, Achermann P, Bischof M, et al. Evolution of sleep and sleep EEG after hemispheric stroke. J Sleep Res 2002; 11:331–338.
51. Landolt H, Glatzel M, Blatter T, et al. Sleep-wake disturbances in sporadic Creutzfeldt_jakob disesae. Neurology 2006; 66:1418–1424.
52. Rumpl E, Prugger M, Bauer G, Gerstenbrand F, Hackl JM, Pallua A. Incidence and prognostic value of spindles in posttraumatic coma. Electroencephalography and Clinical Neurophysiology 1983; 56:420–429.
53. Ron S, Algom D, Hary D, Cohen M. Time-related changes in the distribution of sleep stages in brain injured patients. Electroencephalography and Clinical Neurophysiology 1980; 48:432–441.
54. Hachinski V, Mamelak M, Norris JW. Prognostic value of sleep morphology in cerebral infarction. Amsterdam: (Excerpta Medica); 1979.
55. De Groot MH, Philipps SJ, Eskes GA. Fatigue associated with stroke and other neurologic conditions: Implications for stroke rehabilitation. Arch Phys Med Rehab 2003; 84(11):1714–1720.
56. Palomäki H, Berg AT, Meririnne E, et al. Complaints of poststroke insomnia and its treatment with mianserin. Cerebrovasc Dis 2003; 15:56–62.
57. Scheidtmann K, Fries W, Muller F, Koenig E. Effect of levodopa in combination with physiotherapy on functional recovery after stroke: a prospective, randomized, double-blind study. Lancet 2001; 358: 787–790.
58. Grade C, Redford B, Chrostowski J, et al. Methylphenidate in early poststroke recovery. A double-blind, placebo controlled study. Arch Phys Med Rehab 1998; 79:1047–1050.
59. Teitelman E. Off-label use of modafinil. Am J Psychiatry 2001; 158(8):1341 (letter).
60. Quinto C, Gellido C, Chokroverty S, Masdeu J. Posttraumatic delayed sleep phase syndrome. Neurology 2000; 54:250–252.

31
Potential Mechanisms of the Wake-Promoting Action of Hypocretin/Orexin

MICHAEL MÜHLETHALER
C.M.U. Département de Physiologie, Geneva, Switzerland

I. Introduction

The importance of the hypocretin/orexin (hcrt/orx) system (1,2) in promoting wakefulness has been amply documented in mice, dogs and humans (3–6). The way by which the hcrt/orx system exerts its action is however still a matter of debate and has been our major subject of interest using an in vitro electrophysiological approach that followed essentially two lines. First, we studied the actions of hcrt/orx upon neurons that one could subdivide into three categories: those of the preoptic area associated with promoting sleep, those of major diencephalic structures associated with promoting wakefulness (basal forebrain, tuberomammillary nuclei and intralaminar thalamic nuclei) and finally those of the cerebral cortex. Second, we investigated the intrinsic properties of hcrt/orx neurons that could be relevant to their ability to promote wakefulness.

A. Effects of HCRT/ORX

Sleep-Promoting Neurons

The basal forebrain/preoptic area has been suggested, on the basis of immunohistochemical studies, to contain GABAergic sleep-active neurons (7) which, by their projections, could inhibit wake-promoting neuronal systems throughout the brain and thus enable sleep (for recent review (8)). In a subset of neurons of the ventrolateral preoptic area (VLPO (7)) we have described in vitro a unique population of GABAergic "sleep-promoting" cells (9) that we proposed could correspond to the sleep-active neurons recorded in vivo (10). Such cells were inhibited by the major transmitters of arousal, noradrenaline and acetylcholine (9), including through a presynaptic nicotinic facilitation of noradrenaline release (11). The inhibitory action of these transmitters upon VLPO neurons is thought to contribute to their wake-promoting role. The presence of hcrt/orx fibres (12) and receptors (13) in the VLPO has thus led us to hypothesize that hcrt/orx could also possibly contribute to wakefulness through a direct inhibition of sleep-promoting neurons.

Our study (14) showed however a complete absence of response of VLPO neurons to hcrt/orx and thus does not support this hypothesis. As will be shown below, the excitatory action of hcrt/orx upon systems which themselves act upon sleep-promoting neurons could however provide for an indirect inhibition.

Wake-Promoting Neurons

Hcrt/OX receptors are expressed widely throughout the CNS (13) in correspondence with areas to which hcrt/orx fibers also project (12). Hcrt/OX receptors and fibers are particularly densely distributed in areas known to be involved in cortical activation (reviewed in (8)). Our interest focused upon neurons of the basal forebrain, the tuberomammillary nuclei and the thalamus.

Basal Forebrain Cholinergic Neurons

In contrast to the absence of effect of hcrt/orx on VLPO sleep-promoting neurons, basal forebrain cholinergic neurons were always strongly excited by the peptide (14). This action, which was shown to be postsynaptic, was mediated by Hcrt2/OX_2 receptors as hcrt2/orxB was always more potent than hcrt1/orxA (2). The precise mechanism of action of hcrt/orx has however not yet been explored. These results are in agreement with in situ hybridization studies of Hcrt2/OX_2 receptors being more heavily expressed than Hcrt1/OX_1 receptors in the basal forebrain (13). They are also in agreement with data obtained in vivo that have demonstrated that the local perfusion of hcrt/orx in the basal forebrain increases wakefulness (15). Altogether these data support the notion that the cholinergic system of the basal forebrain, known to be a major system for cortical activation through its widespread projections (8), must represent an important link in the wake-promoting action of hcrt/orx. Of interest in this context was the further demonstration that cholinergic neurons of the mesopontine tegmentum, that are part of a cortical activating system arising from the brainstem, are also targeted by hcrt/orx fibers, express Hcrt/OX receptors and are very sensitive to the peptide (12,13,16).

Tuberomammillary Histaminergic Neurons

Similarly to the action of hcrt/orx on cholinergic neurons, we, and others, found that TMN histaminergic neuron were systematically excited by hcrt/orx and again the action was due to the presence of Hcrt2/OX_2 receptors (17,18). The mechanism of the depolarization following hcrt/orx was shown to depend upon the activation of both a calcium current and an electrogenic sodium/calcium exchanger (17). Given the established implication of histamine in promoting wakefulness (8), these data strongly suggest a role for the TMN in relaying the effect of hcrt/orx. Indeed, the tuberomammillary nuclei with their widespread projections represent the major source of histamine in the brain and when they are inactivated or when the actions of histamine are blocked by specific antagonists, sleep is facilitated (8). Histaminergic neurons are known to be preferentially active during waking and histamine has long been known to exert a strong excitatory action upon neurons in many areas and notably in the thalamus and cerebral cortex (19).

Intralaminar Thalamic Nuclei Neurons

When studying the responsiveness of thalamic nuclei neurons to hcrt/orx, we found that those located in the centromedial (CM) intralaminar and Rhomboide (Rh) midline nuclei were strongly depolarized and excited by the peptide (20). As in the basal forebrain and the tuberomammillary nuclei, these effects are postsynaptic and mediated by Hcrt2/OX_2 receptors. The increased membrane resistance observed during the effect of hcrt/orx and the abolition of the depolarizing effect when the cell is maintained at Ek (equilibrium

potential for potassium) suggest that the action of hcrt/orx is due to the blocking of potassium channels. A similar mechanism has been suggested to explain the depolarizing effect of hcrt/orx upon neurons of the locus coeruleus (21).

An intriguing finding was that neurons in relay nuclei (such as the somatic ventral postero-lateral nucleus or the visual dorso-lateral geniculate nucleus) were, in contrast to the CM and the Rh, totally insensitive to hcrt/orx, although they responded strongly to both noradrenaline (NA) and acetylcholine (Ach). These electrophysiological data are consistent with a lesser presence in these relay nuclei of either Hcrt/OX receptors (13) or hcrt/orx fibers (12).

Our interpretation of these results is that hcrt/orx does not directly influence sensory transmission through the thalamus, but rather promotes widespread cortical activation by acting upon those nuclei that give rise to widespread cortical projections (22,23). These projections include from the intralaminar CM nucleus, the anterior cingulate area, known to be particularly important for arousal, and from the midline Rh nucleus, virtually all cortical areas (24). Interestingly, further studies have shown (25) that hcrt/orx facilitates glutamate release by a presynaptic action upon the very terminals of intralaminar nuclei neurons within the cortex (25), thereby reinforcing in a specific manner the cortical activating role of hcrt/orx.

Cortical Neurons

Our studies of cortical neurons (26) demonstrated that only those neurons of sublayer 6b were excited by hcrt/orx through postsynaptic receptors (Fig. 1). In all other layers (including layer 6b), hcrt/orx had no effect at all, or, in small minority of cells, it provoked a weak increase in spontaneous excitatory synaptic potentials. The direct effect on layer 6b cells was present in all tested cortical areas and thus affected various functional circuits (i.e., parietal somatosensory, frontal motor, occipital visual, and cingulate). As in our previous studies reported above, the excitatory effect of the peptide in the cortex was found to be mediated by Hcrtr2/OX_2 receptors. These data are in agreement with studies showing a higher density of Hcrtr2/OX_2 than Hcrtr1/OX_1 receptors in the cortex and a higher density of the Hcrtr2/OX_2 receptors in the deep than in the superficial layers of the cortex (13). With respect to the mechanism of action, it was shown in the cortex, as in the thalamus, to depend upon the closure of potassium channels (26).

Although neurons in sublayer 6b have not been studied in as much details as neurons in other layers, reports indicate that they have rather widespread cortical projections (27,28). It is in particular noteworthy that they project not only to layer 1, thus targeting the same layer as the thalamic intralaminar nuclei (29), but also to layer 2/3, thereby directly affecting cortico-cortical projections (27,28).

II. Conclusion

Altogether these results indicate that the hcrt/orx system could facilitate wakefulness by either directly activating cortical circuits or by exerting a strong influence on intralaminar/midline thalamic nuclei that have widespread cortical projections, or still by affecting the major activating systems of the brainstem (noradrenergic locus coeruleus, cholinergic mesopontine tegmentum) and the diencephalon (cholinergic basal forebrain and histaminergic tuberomammillary nuclei). With the exception of locus coeruleus

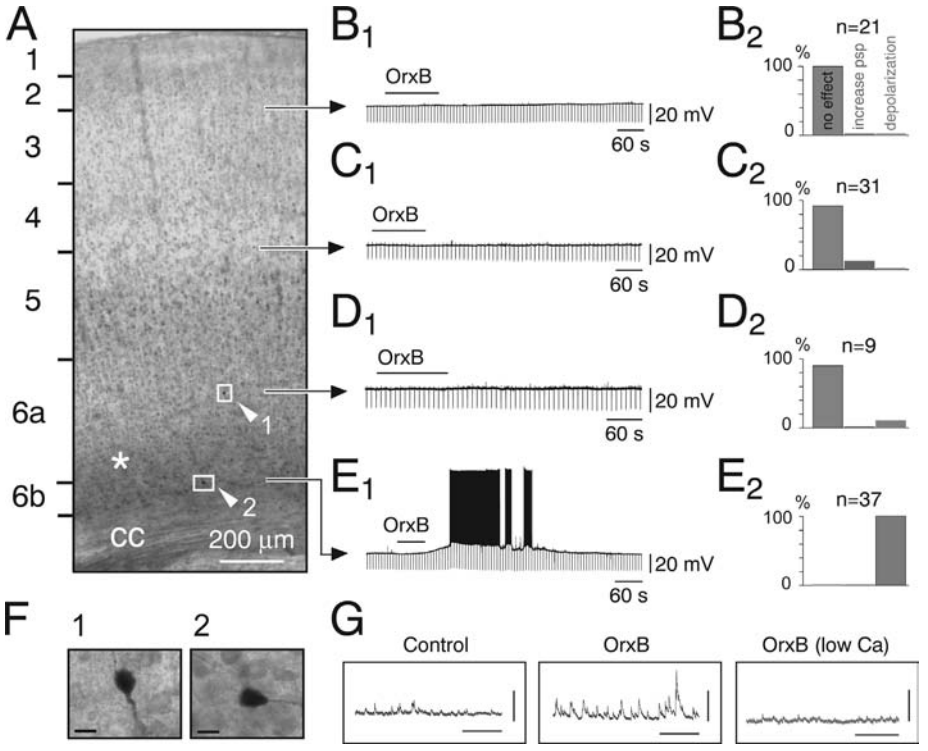


Figure 1 Exclusive action of hcrt/orx on cortical neurons of sublayer 6b in the somatosensory cortex. (***A***) Toluidin blue counter-stained cortical slice slab containing two recorded neurons (arrowheads) labeled with Neurobiotin in sublayers 6a and 6b (separated by a horizontal band of fibers, *) and whose responses to hcrt/orx are shown in panels ***D*** and ***E***. (***B***$_1$) Absence of response to hcrt/orx of a neuron in layer 2/3. (***B***$_2$) Summarized results showing that all neurons of layer 2/3 were unresponsive to hcrt/orx. Number (n) represents the total number of cells tested. (***C***$_1$) Absence of response to hcrt/orx of a neuron in layer 4/5. (***C***$_2$) Summarized results showing that most neurons of layer 2/3 were unresponsive to hcrt/orx (see text). (***D***$_1$) Absence of response to hcrt/orx of a neuron in layer 6a. (***D***$_2$) Summarized results showing that most neurons of layer 6a were unresponsive to hcrt/orx (see text). (***E***$_1$) Depolarizing response to hcrt/orx of a neuron in layer 6b. (***E***$_2$) Summarized results showing that all neurons of layer 6b were depolarized by hcrt/orx. (***F***$_{1-2}$) Enlargement of neurons 1 and 2 from (***A***). *Calibrations*: 15 microm. (***G***) Increase in PSPs in a layer 5 neuron (middle panel) is impeded in presence of a low calcium/high magnesium ACSF. *Calibrations*: 5 mV/2 sec. *Abbreviation*: cc, corpus callosum. *Source*: From Ref. 31.

neurons, whose responses depend upon Hcrtr1/OX$_1$ receptors (30), all the other systems are activated through Hcrtr2/OX$_2$ receptors, which must thus play a major role in maintaining wakefulness. The excitatory action mediated by Hcrtr2/OX$_2$ receptors have been shown to involve several mechanisms, that include activation of an electrogenic pump and calcium currents in histaminergic neurons (17), activation of a nonselective cation current in mesopontine cholinergic neurons (16) and the blocking of a potassium current in thalamic and cortical neurons (20,31).

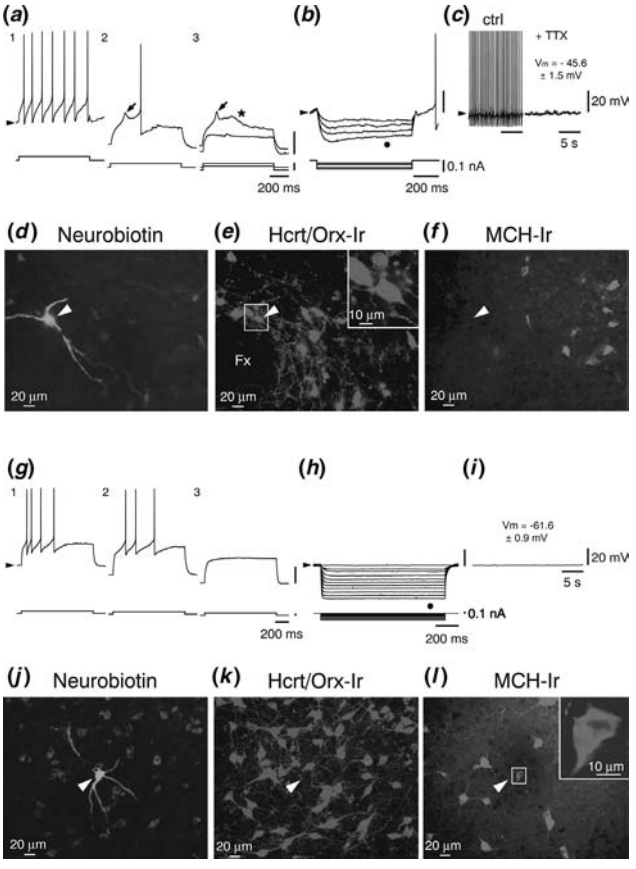


Figure 2 Characterization of neurons expressing hypocretin/orexin or melanin-concentrating hormone. (***a*$_1$**) Tonic firing in response to a depolarizing current pulse delivered from the level of resting potential (*arrowhead*). (***a*$_{2-3}$**) Low-threshold spike (LTS, *arrow*) and after-depolarization (ADP, *star*) triggered by a depolarizing current pulse delivered from an hyperpolarized level. Further hyperpolarization eliminates the LTS and the ADP (lower trace in ***a*$_3$**). (***b***) Superimposed responses to hyperpolarizing pulses suggesting the presence of an I_h current (*dot*). Note that only the trace with the deepest hyperpolarization is shown in full. (***c***) Tonic firing at rest and its elimination by tetrodotoxin (TTX, 1.0 mM) to determine resting potentials. (***d,f***) Immunohistochemical identification of an hypocretin/orexin neuron injected with neurobiotin (*arrow* in ***d***) and expressing immunoreactivity for hypocretin/orexin, Hcrt/OX (***e***) but not for the melanin-concentrating hormone, MCH (***f***). (***g*$_1$**) Firing with accommodation triggered by a depolarizing current pulse delivered from the resting potential level. (***g*$_{2-3}$**) Absence of either LTS or ADP in response to depolarizing pulses applied from more hyperpolarized levels. (***h***) Responses to hyperpolarizing current pulses demonstrating the absence (*dot*) of any sag that could have indicated the presence of an I_h current. (***i***) Absence of spontaneous firing in such neurons and their mean resting potential. (***j,l***) Immunohistochemical identification of a MCH neuron injected with neurobiotin (*arrow* in ***j***) and expressing immunoreactivity for MCH (***k***) but not for hypocretin/orexin, Hcrt/OX (***l***). Membrane potentials (*arrowheads*): −47 mV (***a***), −44 mV (B), −48 mV (***c***), −61 mV (***g***), −61 mV (***h–i***). *Source*: From Ref. 26.

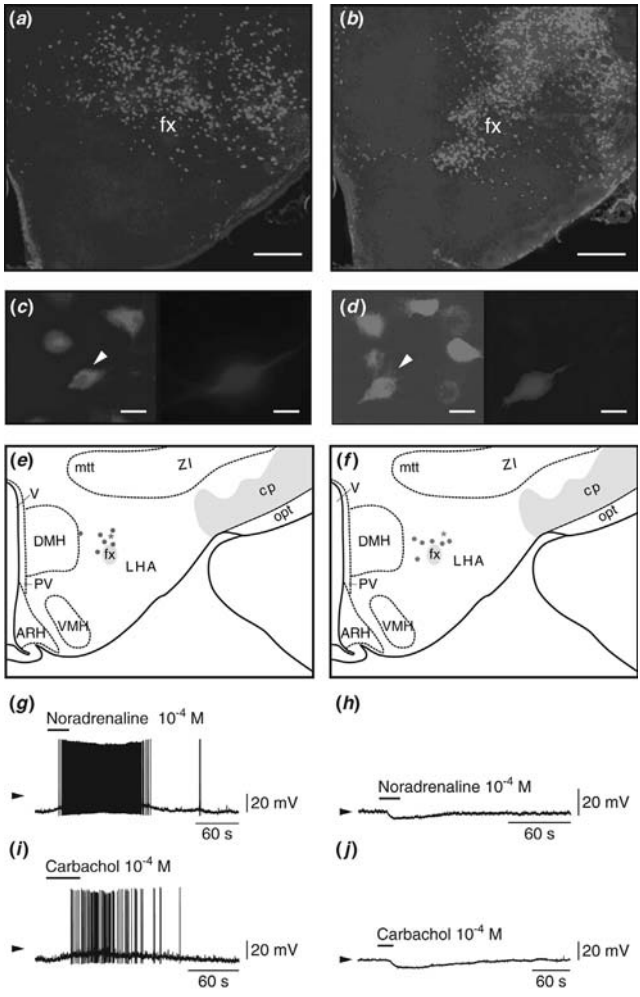


Figure 3 Images of the Hcrt/Orx and MCH cells and their responses to NA and carbachol. (***a–b***) Images of Hcrt/Orx-IR cells (immunostained using red, Cy3-conjugated antiserum in ***a***) and MCH-IR cells (immunostained using green, Alexa Fluor 488-conjugated antiserum in ***b***) in the rat hypothalamic slice. (***c–d***) Nb-labeled neurons (immunostained using blue, AMCA-streptavidin conjugate in right panels) that were immunopositive for Hcrt/Orx (immuno- stained using red, Cy3-conjugated antiserum, left panel in ***c***) or MCH (immunostained using green, Alexa Fluor 488-conjugated antiserum, left panel in ***d***). (***e–f***) Maps through the rat tuberal hypothalamus showing the location of recorded and confirmed Nb-labeled Hcrt/Orx-IR cells (dots in ***e***) and MCH-IR cells (dots in ***f***) upon which noradrenaline (NA) and carbachol, a cholinergic agonist, were tested. (Stars in ***e*** and ***f*** represent the Nb-labeled cells shown in ***c*** and ***d***.) (***g–j***) Whole-cell recordings in current clamp showing responses of confirmed Nb-labeled Hcrt/Orx-IR cells (in ***g*** and ***i***) and MCH-IR cells (in ***h*** and ***j***) to NA (***g–h***) and to carbachol (***i–j***). Magnification bars = 0.1 mm in ***a–b*** and 20 mm in ***c–d***. Resting membrane potential in ***g–j*** is indicated by arrowheads. *Source*: From Bayer et al. (2005) (in press).

A. Intrinsic Properties and Modulation of HCRT/ORX Neurons

By comparing immunohistochemically identified hcrt/Orx neurons and the codistributed melanin-concentrating hormone (MCH) neurons (Fig. 2), we found that hcrt/orx neurons, in contrast to MCH neurons, were in a depolarized and active state (26). Our study indicated that this state should depend upon to the intrinsic properties of the cells as it persisted in the absence of synaptic transmission. As hcrt/orx cells were found to be endowed with a strong calcium-activated nonselective cation current (32), and after excluding possible contributions from other currents, we speculated that this current could play an important role in the persistent membrane depolarization.

B. Conclusion

During wakefulness, the enduring activity of hcrt/orx neurons reported above should allow them to provide a tonic excitatory drive to their multiple targets that include the cortex, the intralaminar/thalamic nuclei and the major activating systems discussed previously. By such a coordinated action on these multiple systems, these neurons could play a central in promoting and maintaining the state of wakefulness. Interestingly, in a recent report we have demonstrated that both noradrenaline and acetylcholine, while inhibiting MCH neurons, are exciting hcrt/orx neurons (Fig. 3), thus providing for a positive feed-back that could play an important role in maintaining the overall activity of the activating systems during wakefulness {Bayer, 2004 #779}. Of final interest, is the question of the way by which hcrt/orx neurons could, possibly through an active inhibition, decrease or cease their activity during quiet waking or sleep (BE Jones, personal communication). As the presence of $GABA_A$ (26) and $GABA_B$ dependant responses (L. Bayer and BE Jones, personal communication) have been identified upon hcrt/orx neurons, it is probable that the GABAergic neurons of the preoptic area, which are known to become active during sleep, could provide for such inhibition (7,9,10).

Acknowledgment

Supported by grants from the Swiss Funds National.

References

1. de Lecea L, Kilduff TS, Peyron C, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 1998; 95(1):322–327.
2. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92(4):573–585.
3. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27(3):469–474.
4. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355(9197):39–40.
5. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6(9):991–997.
6. Hara J, Beuckmann CT, Nambu T, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001; 30(2):345–354.

7. Sherin JE, Elmquist JK, Torrealba F, Saper CB. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 1998; 18(12):4705–4721.
8. Jones BE. Arousal systems. Front Biosci 2003; 8:S438–S451.
9. Gallopin T, Fort P, Eggermann E, et al. Identification of sleep-promoting neurons in vitro. Nature 2000; 404(6781):992–995.
10. Szymusiak R, Alam N, Steininger TL, McGinty D. Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. Brain Res 1998; 803(1–2):178–188.
11. Saint-Mleux B, Eggermann E, Bisetti A, et al. Nicotinic enhancement of the noradrenergic inhibition of sleep-promoting neurons in the ventrolateral preoptic area. J Neurosci 2004; 24(1):63–67.
12. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18(23):9996–10015.
13. Marcus JN, Aschkenasi CJ, Lee CE, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001; 435(1):6–25.
14. Eggermann E, Serafin M, Bayer L, et al. Orexins/hypocretins excite basal forebrain cholinergic neurones. Neuroscience 2001; 108(2):177–181.
15. Methippara MM, Alam MN, Szymusiak R, McGinty D. Effects of lateral preoptic area application of orexin-A on sleep-wakefulness. Neuroreport 2000; 11(16):3423–3426.
16. Burlet S, Tyler CJ, Leonard CS. Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implications for wakefulness and narcolepsy. J Neurosci 2002; 22(7):2862–2872.
17. Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 2001; 21(23):9273–9279.
18. Bayer L, Eggermann E, Serafin M, et al. Orexins (hypocretins) directly excite tuberomammillary neurons. Eur J Neurosci 2001; 14(9):1571–1575.
19. McCormick DA, Bal T. Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci 1997; 20:185–215.
20. Bayer L, Eggermann E, Saint-Mleux B, et al. Selective action of orexin (hypocretin) on nonspecific thalamocortical projection neurons. J Neurosci 2002; 22(18):7835–7839.
21. Ivanov A, Aston-Jones G. Hypocretin/orexin depolarizes and decreases potassium conductance in locus coeruleus neurons. Neuroreport 2000; 11(8):1755–1758.
22. Groenewegen HJ, Berendse HW. The specificity of the "nonspecific" midline and intralaminar thalamic nuclei. Trends Neurosci 1994; 17(2):52–57.
23. Herkenham M. Laminar organization of thalamic projections to the rat neocortex. Science 1980; 207(4430):532–535.
24. Berendse HW, Groenewegen HJ. Restricted cortical termination fields of the midline and intralaminar thalamic nuclei in the rat. Neurosci 1991; 42(1):73–102.
25. Lambe EK, Aghajanian GK. Hypocretin (orexin) induces calcium transients in single spines postsynaptic to identified thalamocortical boutons in prefrontal slice. Neuron 2003; 40(1):139–150.
26. Eggermann E, Bayer L, Serafin M, et al. The wake-promoting hypocretin-orexin neurons are in an intrinsic state of membrane depolarization. J Neurosci 2003; 23(5):1557–1562.
27. Reep RL. Cortical layer VII and persistent subplate cells in mammalian brains. Brain Behav Evol 2000; 56(4):212–234.
28. Clancy B, Cauller LJ. Widespread projections from subgriseal neurons (layer VII) to layer I in adult rat cortex. J Comp Neurol 1999; 407(2):275–286.
29. Herkenham M. New perspectives on the organization and evolution of nonspecific thalamocortical projections. In: Jones EG, Peters A, eds. Cerebral cortex. New-york: Plenum; 1986:403–445.
30. Bourgin P, Huitron-Resendiz S, Spier AD, et al. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. J Neurosci 2000; 20(20):7760–7765.
31. Bayer L, Serafin M, Eggermann E, et al. Exclusive postsynaptic action of hypocretin-orexin on sublayer 6b cortical neurons. J Neurosci 2004; 24(30):6760–6764.
32. Partridge LD, Muller TH, Swandulla D. Calcium-activated non-selective channels in the nervous system. Brain Res Brain Res Rev 1994; 19(3):319–325.

32
Normal Role of Hypocretin/Orexin

JEROME M. SIEGEL
Neurobiology Research, VA GLAHS Sepulveda, UCLA Department of Psychiatry, David Geffen School of Medicine, Los Angeles, California, U.S.A.

I. Introduction

There is now no doubt that hypocretin/orexin (Hcrt) deficiency is the cause of most narcolepsy in humans (1,2). It is also clear that mutations of the Hcrt system cause genetic narcolepsy in several animal species (3,4). Narcolepsy in these Hcrt deficiency situations is characterized by sleepiness during the normally active period and losses of muscle tone during waking periods called cataplexy. We know from observations in humans and in canine narcoleptics that such cataplectic episodes are accompanied by unimpaired consciousness, and it is reasonable to expect that this is also the case in rodent Hcrt mutant models.

These findings lead to the conclusion that one of the functions of Hcrt is to prevent the symptoms of narcolepsy. In this chapter I will address the question of how this function of Hcrt might be achieved and consider evidence bearing on a more general role of Hcrt in behavioral control. It is unlikely that simply defining the differences in behavior between narcoleptic and normal individuals will illuminate all of the functions of Hcrt. It is virtually certain that a variety of brain systems compensate for the postnatal loss of Hcrt in humans by occupying vacated synaptic sites and by up and down regulation of receptors whose activity is altered by the loss of Hcrt. It is also likely that Hcrt mutants have developmental alterations that modify, and perhaps ameliorate, the effects of Hcrt mutations, thus masking some of the normal functions of Hcrt.

An effective alternate strategy for determining the role of Hcrt is to observe fluctuations in its release in normal animals, in which the role will not be obfuscated by compensatory reorganization. Such observations, including studies of release using microdialysis and cerebrospinal fluid (CSF) assays, will indicate when this peptide is released. Ultimately, it may be possible to bridge the gulf between information gained from such observations and the pathology of narcolepsy, by better understanding the neurological adjustments that result form Hcrt dysfunction.

One early theory of Hcrt function was that it was an "orexigenic" or appetite-stimulating compound. This was based on initial observations that high doses of Hcrt injected into the lateral hypothalamus, a region whose stimulation is known to induce feeding, increased food intake. However, subsequent infusion studies did not always support these initial observations (5). We tested the hypothesis that

Hcrt was involved in the regulation of food intake by studying the release of Hcrt in dogs under baseline conditions, after 48 hours of food deprivation and at various times after feeding (6). To our surprise, we found that these manipulations had very small effects that were statistically insignificant. However, when these same normal animals were sleep deprived for as little as 24 h, Hcrt levels increased by an average of >70%. Further analysis showed that it was not the sleep loss per se that drove the Hcrt level up. Rather, the activity that was induced to prevent sleep, as measured by actigraphs worn by the dogs, was most tightly correlated with the rise in Hcrt level. We followed up on this finding by increasing motor activity with vigorous play with the animals for up to 2 hours. This again produced a 60% to 70% increase in Hcrt level compared to levels in the same animals during alert waking, with the increase in individual trials correlated with activity level. We conclude from these studies that, in contrast to food deprivation and consumption, motor activity is tightly correlated with Hcrt release (6,7).

This finding is compatible with what we know about the connections of Hcrt cells. The most massive extrahypothalamic projection of the Hcrt system is to the locus coeruleus.[5] We found that microinjection of Hcrt into the locus coeruleus produced a striking increase in muscle tone (7). In other work, we showed that locus coeruleus activity is tightly linked to muscle tone, and that in narcoleptic dogs locus coeruleus cells cease discharge immediately prior to and during cataplexy (8). The locus coeruleus facilitates muscle tone and a group of cells in the medial medulla inhibit muscle tone. These two systems are tightly and reciprocally linked (9). One can therefore see that loss of Hcrt will interrupt a pathway that increases muscle tone by direct facilitation and simultaneous disinhibition.

One can explain cataplexy, even if one does not invoke potential neural reorganizations in Hcrt deficient animals, by hypothesizing that Hcrt neurons are normally activated phasically during certain emotional stimuli to maintain muscle tone. In the absence of this compensatory excitation, the underlying reduction in tone accompanying strong emotional stimuli is revealed. In humans, the most common triggers for cataplexy: laughter, anger and surprise are consistently accompanied by motor changes even in normal individuals, as exemplified by "doubling over with laughter," becoming speechless or stammering with anger, dropping objects when surprised or shocked, and so on. In narcoleptics with cataplexy, these same conditions elicit complete, rather than partial, suppressions of muscle tone.

Our findings and other studies that show a strong relationship between motor activity and Hcrt release are certainly not the last word in the analysis of the behavioral role of Hcrt. Although it appears clear that Hcrt release is elevated during motor activity, many questions remain. Is Hcrt related to all movements or only to particular types of movements? For example, do Hcrt levels increase to the same extent in rhythmic movements such as grooming as they do in exploration? Is Hcrt release associated with, for example, head vs. limb movements? Is Hcrt release more tightly linked to ipsilateral vs. contralateral movements? Does Hcrt level increase in relation to movement intensity? Is Hcrt release related to the emotional aspects or to cognitive changes not present in quiet waking but characterizing motorically active states? Such questions can best be answered by recording from identified Hcrt neurons in freely moving animals, a challenge that has not yet been met.

Whatever the fine-grained analysis of Hcrt activity and motor activity reveals, we do know that Hcrt can act directly on motoneurons. Hcrt neurons project to motoneurons, and when Hcrt is microinjected into motoneuron pools it produces a profound motor excitation. However, this excitation is completely blocked by glutamate antagonists (10). This indicates that Hcrt acts through glutamate at the motoneuronal level. It is possible that Hcrt modulation of glutamate release may be a major mode of Hcrt action. We have shown that intravenous injection of Hcrt produces release of glutamate in Hcrt innervated regions as exemplified by the amygdala, but not in regions not innervated by Hcrt, as exemplified by the cerebellum (11).

Apart from its facilitation of motor systems and its correlated activation of forebrain waking systems, Hcrt is likely to function in the coordination of monoaminergic and cholinergic systems. Individual Hcrt neurons have projections to multiple aminergic systems, strongly suggesting a coordination role (5).

A key effect of the lack of coordination of monoaminergic cells by Hcrt deficiency can be seen in cataplexy. In narcoleptic dogs, whose cataplexy attacks are behaviorally and pharmacologically indistinguishable from those in human narcolepsy, a striking dyscoordination of monoaminergic activity is seen. In the normal animal, histamine and norepinephrine cells are both tonically active throughout waking. Arousing stimuli activate these cell groups and there is some reduction in activity during quiet waking. In sleep, both cell groups cease activity. A striking finding is that, although these two cell groups increase and decrease discharge more or less simultaneously in all waking-sleep states in normal animals, they do not do this in narcoleptic animals. We have recently shown that in cataplexy, histamine cells increase activity, as might be expected in normal animals during excitement, but norepinephrine cells completely cease activity (12). Thus, either through the direct action of Hcrt on norepinephrine and histamine neurons, or through neurological reorganization subsequent to Hcrt dysfunction, this coordinated activity is lost, resulting in the dissociated firing of norepinephrine and histamine cells that is linked to cataplexy. We hypothesize that the loss of norepinephrine release causes the loss of muscle tone of cataplexy, whereas the maintained activity of histamine cells produces continued consciousness. In contrast the cessation of activity in both of these cell groups results in sleep, with a correlated reduction of muscle tone.

II. Conclusion

Much remains to be done in order to elucidate the role of Hcrt in normal behavior. The Hcrt system interacts closely with monoaminergic and amino acid systems. A link to motor activity is clear, and a link to certain types of motivated behaviors is likely, but the precise relationship underlying these links is not yet understood.

Acknowledgments

Supported by, PHS grants NS14610, MH64109, HL060296 and the Medical Research Service of the Dept. of Veterans Affairs. Additional references can be found at: http://www.npi.ucla.edu/sleepresearch

References

1. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat.Med.. 2000; 6:991–997.
2. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
3. Lin L, Faraco J, Kadotani H, Rogers W, Lin X, Qui X, et al. The REM sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor gene. Cell 1999; 98:365–376.
4. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, et al. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron JID - 8809320 2003; 38:715–730.
5. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. Annual Rev.of Psychol. 2004; 55:125–148.
6. Wu MF, John J, Maidment N, Lam HA, Siegel JM. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. Am0 J Physiol Regul Integr Comp Physiol 2002; 283:R1079–R1086.
7. Kiyashchenko LI, Mileykovskiy BY, Lai YY, Siegel JM. Increased and decreased muscle tone with orexin (hypocretin) microinjections in the locus coeruleus and pontine inhibitory area. J Neurophysiol. 2001; 85:2008–2016.
8. Wu MF, Gulyani S, Yao E, Mignot E, Phan B, Siegel JM. Locus coeruleus neurons: cessation of activity during cataplexy. Neuroscience 1999; 91:1389–1399.
9. Mileykovskiy BY, Kiyashchenko LI, Kodama T, Lai YY, Siegel JM. Activation of pontine and medullary motor inhibitory regions reduces discharge in neurons located in the locus coeruleus and the anatomical equivalent of the midbrain locomotor region. J Neurosci 2000; 20:8551–8558.
10. Peever JH, Lai YY, Siegel JM. Excitatory Effects of Hypocretin-1 (Orexin-A) in the Trigeminal Motor Nucleus Are Reversed by NMDA Antagonism. J Neurophysiol. 2003; 89:2591–2600.
11. John J, Wu M-F, Kodama T, Siegel JM. Intravenously administered hypocretin-1 alters brain amino acid release: an *in vivo* microdialysis study in rats. J.Physiol.(Lond). 2003; 548.2:557–562.
12. John J, Wu M-F, Boehmer LN, Siegel JM. Cataplexy-active neurons in the posterior hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 2004; 42:619–634.

33
Role of Hypocretin/Orexin in the Neurobiology of Sleep and Alertness

JAMIE M. ZEITZER and EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Hypocretin Neurobiology

There are two known, biologically active hypocretins, hypocretin-1 (orexin A) and hypocretin-2 (orexin B), that are proteolytically derived from preprohypocretin (prepro-orexin) (1,2). In the brain, hypocretins are only produced in a small, scattered group of neurons in the lateral hypothalamus. These neurons have projections throughout the brain, including forebrain, midbrain, hindbrain, and spinal cord (3–5). Notably, hypocretin-containing axons project to nuclei that are integral to the regulation of sleep and wake. Additionally, hypocretin-containing axons innervate areas involved regulating wake-associated functions such as locomotion, sympathetic regulation, and feeding.

Hypocretin-recipient areas of the brain express one or both of the hypocretin receptors, hypocretin receptor 1 (hcrtr1, orexin receptor 1) and hypocretin receptor 2 (hcrtr2, orexin receptor 2) (6,7). Hcrtr1 is more selective for hypocretin 1 than for hypocretin 2, while hcrtr2 is equally selective for both of the hypocretins. Activation of the hypocretin receptors leads to presynaptic and/or postsynaptic excitation through a variety of target tissue-specific second messenger and ionic systems (e.g., GIRK channel activation in the locus coeruleus, sodium-calcium exchanger in the arcuate nucleus) (8,9). Although the exact roles of the two receptors have not yet been fully elucidated, differences have been noted. Hcrtr2 seems to be more important in the regulation of wakefulness as mice lacking hcrtr2 have a more disrupted wake than mice lacking hcrtr1 (10). However, hcrtr1 is still likely to be important in controlling wakefulness, especially transitions from wake to REM sleep, since the loss of both hypocretin receptors produces an even more severe disruption of wake than the loss of hcrtr2 alone (10).

II. Effects of Exogenously Applied Hypocretins

Administration of exogenous hypocretin-1 or -2 into the cerebrospinal fluid (CSF) of the cerebral ventricles (i.c.v.) of rodents can cause a short-term increase in wakefulness (Fig. 1, 11–14). Such administration will also cause a short-term increase in the concomitants of wakefulness (e.g., locomotion, feeding, drinking, and sympathetic

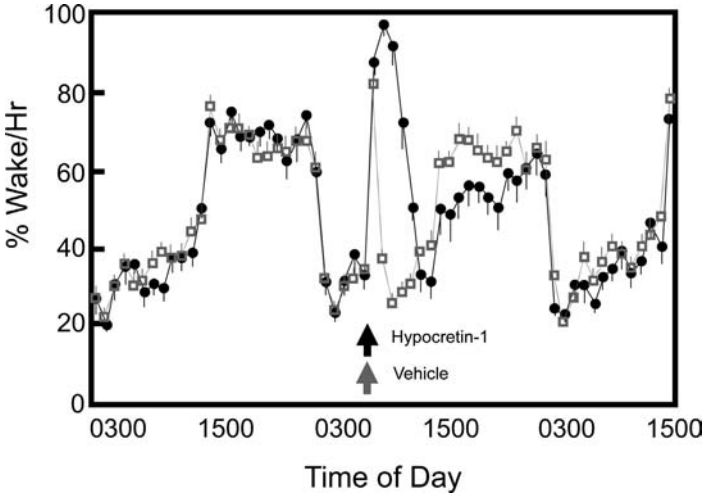


Figure 1 Injection of hypocretin-1 (8 nmoles) into the lateral ventricle of a rat causes an acute increase in wakefulness. This is followed by a compensatory rebound sleep. *Source*: Courtesy of S. Wurtz, Stanford University.

activity). Over a 24-hr period, however, there is not a cumulative increase in these behaviors, as the hypocretin-induced increases are typically followed by a compensatory decrease. The question remains whether hypocretin administration directly causes the increase in wake-related behaviors or if it acts indirectly via an increase in wakefulness that then increases these behaviors. One study supports the latter theory (15), however, given the diverse projections of the hypocretin system, it seems more likely that hypocretin is stimulating both the state of wakefulness and the physiologic mechanisms that accompany this state. As hypocretin administration induces wake that is followed by a compensatory rebound, hypocretin may only be able to influence the likelihood of the occurrence of wake, but not the homeostatic need for sleep. As such, endogenous hypocretin is most likely involved in modulating the timing of wake-related behaviors, as opposed to directly driving the appetitive needs of these behaviors. Studies in humans lacking hypocretin (narcoleptics) (16,17) indicate that hypocretin may, more specifically, be involved in the promotion of wakefulness late in the day.

III. Downstream Mediators of Hypocretin Effects on Wakefulness

Potential mediators of hypocretins' wake stimulating effects include the tuberomammillary nucleus (TMN, histamine), raphe nucleus (serotonin), locus coeruleus (noradrenaline), ventral tegmental area (dopamine), and lateral dorsal tegmentum/pedunculopontine tegmentum and basal forebrain (acetylcholine). Each of these structures receives a robust, excitatory innervation from hypocretinergic axons, express one or both of the hypocretin receptors, and has been implicated in the control of sleep and

wake behavior. Direct application of hypocretins onto many of these structures leads to an increase in wakefulness (e.g., locus coeruleus (18) and lateral dorsal tegmentum/pedunculopontine tegmentum (19)). The breadth of innervation of hypocretin-containing axons lends further credence to the idea that hypocretins may not only increase wakefulness *per se*, but also behaviors and physiology that are specific for the wake state, as described above.

As activation of the various neuromodulator systems may modulate various aspects of wake-related behavior, the question arises as to the system or systems that are involved in the mediation of hypocretin's wake state-promoting function. Histamine appears to be a key neurotransmitter system in this wake-inducing activity of hypocretin. Histaminergic neurons are located in the TMN, receive innervation from hypocretin-containing axons (20), and express mostly hcrtr2 (7,20). As mentioned above, hcrtr2 is likely more important in mediating the wake-related functions of hypocretin than is hcrtr1. Hypocretin is excitatory in the TMN causing both an increase in TMN firing rate (20,21) and a subsequent increase in the release of histamine in a variety of target tissue, including the hypothalamus and cortex (22,23). Pharmacologically blocking the histamine H1 receptor (20) or genetically ablating this receptor (23) precludes or attenuates the wake-promoting effects of i.c.v. administration of hypocretin-1. This implies that i.c.v. hypocretin-1 is able to increase wakefulness via activation of histaminergic neurons. It is quite possible, therefore, that endogenous hypocretin may act primarily through the histaminergic system to increase wakefulness.

IV. CSF Hypocretin-1 as a Useful Proxy of Hypocretin Release

Hypocretin-1, as examined by *in vivo* microdialysis of rat lateral hypothalamus, exhibits a slow diurnal variation, with peak concentrations occurring during the "wake" period and a nadir occurring in the middle of the "sleep" period (Fig. 2a). A similar temporal pattern is observed in CSF hypocretin-1 concentrations measured in the cerebellomedullary cistern (cisterna magna) of rats (Fig. 2a,b), indicating that measuring hypocretin-1 in cisternal CSF is a useful proxy for cerebral hypocretin-1 release.

Other techniques such as *in situ* hybridization of preprohypocretin mRNA (Fig. 2g) or measuring Fos in hypocretin cells (Fig. 2h), or assessment of tissue peptide content (Fig. 2e,f), have also been used to examine the hypocretin system. Use of Fos is sometimes problematic as there can be a mismatch between Fos activation and peptide release (24), an effect we have observed with CSF hypocretin-1 and Fos as well (Zeitzer et al., unpublished results). Fos is, typically, a better marker of cellular responsiveness to unusual stimuli, rather than basal functioning. Examination of preprohypocretin mRNA is useful to examine long-term regulatory changes in gene expression, but there is a long, unknown delay between a change in transcription levels and a change in the release of a peptide. Determination of tissue peptide levels is especially useful to examine hypocretin-2, as the biologic instability of this molecule makes it difficult to measure in the CSF, but the signal-to-noise ratio in these studies is often poor. Measuring tissue peptide content is also likely to reflect long-term neurotransmitter storage rather than actual release. It is notable that hypothalamic hypocretin-1 content is antiphase with release, suggesting important

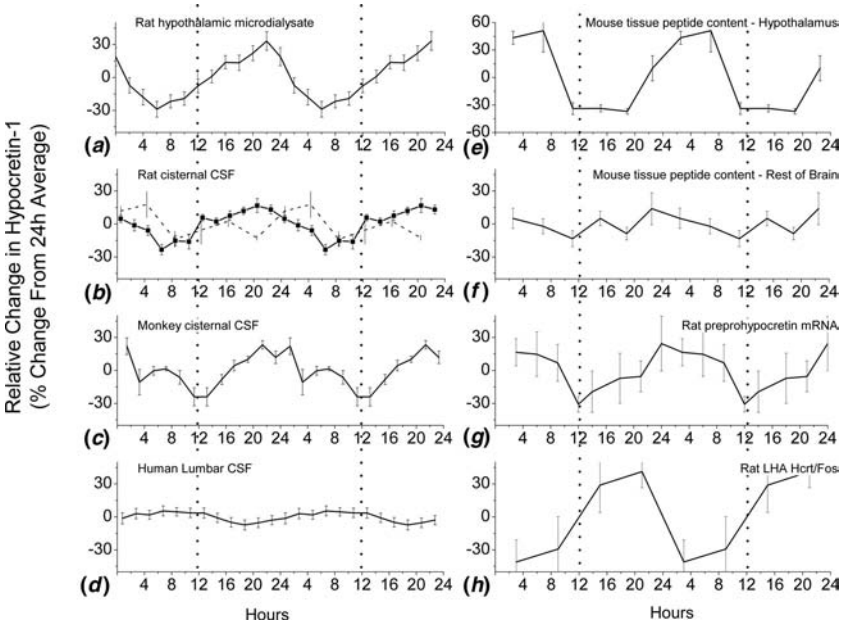


Figure 2 Hypocretin-1 peptide (***a-f***), preprohypocretin mRNA (***g***), and Fos expression in hypocretin neurons (***h***) exhibit a robust daily variation. Data have been normalized to the 24-hour average and aligned according to time of activity onset (hour 12, *dotted line*). Note the various sources and techniques used for assay. The dashed line in ***b*** represents data from rats with lesioned SCN. All data were collected while animals lived in a light:dark cycle. Data in ***a-f*** were obtained in our laboratory and ***g*** and ***h*** were derived from graphically published data. *Sources*: a: Ref. 39; b: controls, Ref. 34, SCN lesion, Ref. 25; c: Ref. 45; d: Ref. 36; e: Zeitzer, Lin, Mignot, unpublished data; f: Zeitzer, Lin, Mignot, unpublished data; g: Ref. 57; h: Ref. 58.

regulation in the trafficking from cell bodies to terminals. These three techniques are also limited in that they all require the study animal to be euthanized, which prevents repeated measures testing in a single animal.

Overall, we believe that *in vivo* microdialysis or direct electrophysiological recording of hypocretin neurons gives the best temporal and spatial resolution of all the techniques. These two techniques are also most likely to reflect actual neurotransmitter release. Direct electrophysiological measurements are, however, difficult and time consuming in non-anesthetized or freely moving animals. *In vivo* dialysis, too, is limited in that peptide concentrations cannot be compared between animals or even between probes in the same animal. Cisternal sampling of CSF does not yield useful spatial release information, but yields general information about hypocretin tone. It is also easy to perform. Thus far, microdialysis and cisternal sampling have proven the most useful techniques to study the relationships between behavior and hypocretin.

V. Diurnal Fluctuation and Circadian Regulation of Hypocretin Release

In rats, the temporal pattern of cisternal CSF hypocretin-1 is dependent upon the suprachiasmatic nuclei (SCN), the location of the mammalian circadian clock. Ablation of the SCN results in an arrhythmic pattern of cisternal CSF hypocretin-1 concentrations (Fig. 2b, dashed line). This arrhythmicity of hypocretin-1 is observed in SCN-ablated rats kept under constant light or constant dark conditions, as well as in these rats kept in a light/dark cycle (12 hours light/12 hours dark) (25). Importantly, we observed that the dynamic range of hypocretin-1 was the same in SCN lesioned rats and rats with intact SCN. A preliminary report of another study, however, indicated that hypocretin-1 concentrations were lower in SCN lesioned rats than in sham-operated controls (26). Thus, while the SCN are necessary for the rhythmic expression of hypocretin-1, it may only temporally gate excitatory and inhibitory inputs to hypocretin neurons, if the dynamic range of hypocretin concentrations is the same in sham-operated and SCN-ablated rats. SCN control of hypocretin neurons may be via direct (27) or indirect (28) innervation, though there is only sparse monosynaptic connectivity between the nuclei.

We also have examined the diurnal variation of hypocretin-1 in CSF continuously collected from the lumbar sac of healthy humans (Fig. 2d). While lumbar CSF concentrations of hypocretin-1 have a diurnal rhythm, the amplitude is quite small (6%) and peak concentrations are observed late in the night (2-4 hours before activity onset). This pattern is quite distinct from that observed in the diurnal squirrel monkey (*Saimiri sciureus sciureus*) which has peak cisternal CSF concentrations of hypocretin-1 occurring late in the active period (*cf.*, Fig. 2c). The discrepancy between these two findings may be due to an extended equilibration delay between peptide release in the brain and accumulation in the human lumbar sac (29). Alternatively, lumbar sac concentrations of hypocretin-1 may be more representative of spinal release of hypocretin-1 (3) than of cortical release (as would be detected in the cisterna magna). A final possibility is that humans have a fundamentally different regulation of their hypocretin system than do rats or squirrel monkeys.

VI. Variable Effects of Stress, Locomotion and Manipulation of Food Intake on Hypocretin Release

A purely circadian-driven alertness signal would not be affected by changes in sleep/wake state or changes in other behaviors. Evidence indicates this is, however, not the case for the hypocretin system. The lateral hypothalamus is innervated by a wide variety of systems, including the limbic forebrain, subcortical visual areas, subthalamus, brain stem, and other hypothalamic nuclei, all of which can convey a variety of information about the external and internal milieus (30). Hypocretin neurons in the lateral hypothalamus appear to react to information conveyed by these systems concerning the state of metabolic homeostasis. Injection of NPY into the lateral hypothalamus induces an increase in Fos expression in hypocretin neurons and a concomitant increase in both feeding and drinking in rats (31). Hypocretin neurons are innervated

by neuropeptide Y (NPY) axon terminals (32) and express NPY-specific Y4 receptors (31). Circulating hormones that relay metabolic-state information can also affect hypocretin neurons. Appetite enhancers, such as ghrelin and decreasing concentrations of glucose, cause an activation of hypocretin neurons, while appetite suppressors, such as leptin and increasing concentrations of glucose, inhibit hypocretin neurons (33). Hypocretin neurons also receive a robust innervation from agouti-related peptide- (appetite enhancer) and melanocyte stimulating hormone (appetite suppressor)-containing axons (32), though the physiologic significance of these connections has not yet been described.

While the anatomic and electrophysiologic data indicate metabolic status can influence the activity of hypocretin neurons, the existing behavioral physiology indicates that this influence is limited to a particular set of conditions. Depriving a rat (34) or dog (35) of food for less than two days does not significantly affect cisternal CSF concentrations of hypocretin-1. We have also observed that a brief ($<$12 hour) food deprivation in a group of squirrel monkeys ($n = 8$) has no affect on cisternal CSF concentrations of hypocretin-1 (avg $\pm$ sem: 7.5% $\pm$ 7.3%; $p = 0.54$, paired T-test) (Zeitzer et al., unpublished observations). Feeding also does not seem to have a significant impact on hypocretin-1. Refeeding after food deprivation does not significantly change cisternal CSF hypocretin-1 concentrations in dogs (35). Likewise, there were no apparent effects of meals on continuously collected lumbar CSF concentrations of hypocretin-1 in humans (36). While shorter duration food deprivation and feeding do not appear to have a large impact on the hypocretin system, extended food deprivation does seem to exert an influence. Three days of food deprivation in rats causes a 34% increase in cisternal CSF hypocretin-1 concentrations (34). While more experimentation is needed, it appears as if the hypocretin system is more responsive to long-term changes in metabolic status than it is to immediate metabolic conditions.

The hypocretin system, as discussed above, is able to modulate sleep/wake status and locomotor rhythms. One of the functions of the hypocretin system may, therefore, be to change the pattern of wakefulness and locomotion in response to long-term changes in food availability. When faced with decreased food availability, two general strategies have been observed in many animals. Some animals (e.g., deer mice) reduce their overall locomotor levels in response to food restriction, presumably to lower their overall energy requirements (37). Other animals (e.g., house mice) change the temporal organization, but not the overall amounts, of their locomotor and wake activity, presumably to change the timing of food exploration (37). In fact, some paradigms of food restriction can even cause a nocturnal animal to have daytime rather than nighttime activity, though the phase of the circadian oscillator remains unchanged (38). Given the unique inputs and outputs of the hypocretin system and the specific response of this system to extended food restriction, it is compelling to hypothesize that the hypocretin system is responsible for altering locomotor and wake patterns to fit long-term changes in food availability, such as may occur seasonally. Future studies will be necessary to test the validity and extent of this hypothesis.

Locomotion is another wake-related behavior that is likely modulated by the hypocretin system. In order to investigate the relationship between basal locomotion and hypocretin tone, we examined cisternal CSF concentrations of hypocretin-1 in rats that had their SCN ablated and that were kept in constant darkness. These conditions removed the robust confounds of SCN-induced variation in locomotor behavior

(as the SCN-mediated changes in hypocretin would co-vary) as well as the locomotor suppressing effects of light (masking). Under these conditions we found a moderate correlation between locomotor activity and hypocretin-1 concentrations ($r = 0.64$, $p < 0.0001$) (25). Locomotor activity that occurred 3-4 hours before collection of CSF had the best predictive value for cisternal hypocretin-1 concentrations. As the time lag between hypothalamic release of hypocretin-1 (39) and cisternal CSF concentrations of hypocretin-1 (34) is likely less than 1 hour in the rat, and locomotion occurring 3-4 hours before CSF was best correlated with hypocretin-1, locomotion in the rat is likely to be feeding back onto the hypocretin system. We have also found that locomotor activity occurring 45-77 minutes prior to collection of CSF in squirrel monkeys was moderately, but significantly ($r = 0.46$, $p < 0.05$) correlated with cisternal hypocretin-1 concentrations (40).

To more directly address the issue of directionality, the effects of forced locomotion on cisternal CSF concentrations of hypocretin-1 has been examined in rats, dogs, and squirrel monkeys. In rats and dogs, forced locomotion elicited an increase in hypocretin-1 (35,41). Forcing locomotion in rats and dogs, however, causes a concomitant change in wakefulness that can independently affect hypocretin tone (see below) (i.e., due to the unconsolidated nature of sleep in these species, forcing movement also forces wakefulness). In squirrel monkeys, a species that remains continuously awake for 12-16 hours per day without stimulation (as do humans), forced locomotion of 161% over baseline for 3 hours causes a non-significant increase in cisternal CSF hypocretin-1 ($39\% \pm 15\%$, $p = 0.07$, paired T-test) (40).

The physiologic pathway by which locomotion feeds back onto the hypocretin system is still unknown. Norepinephrine, dopamine, and direct neural feedback are all possibilities, and are not mutually exclusive. A preliminary study in cats may become particularly informative on this issue (42). In this study, animals spent 60-90 minutes in a specific behavior, including spontaneous wakefulness and forced treadmill walking, and then were euthanized so the expression of Fos in hypocretin neurons could be quantified. While there was a greater amount of locomotor activity in the treadmill condition than in the spontaneous locomotor condition, there was a significantly greater increase in Fos expressing hypocretin neurons in the latter. This implies that the positive feedback from locomotion may be mediated through reward systems (e.g., dopamine) rather than a neural quantification of the activity itself.

Why does locomotion robustly affect the hypocretin system in one group of animals but only slightly in another? The answer may lie in the fact that rats and dogs, as well as mice, cats, Degus, and most other species that have been used to examine the normal physiology of the hypocretin system, are polyphasic with regards to sleep. In other words, instead of consolidating wake and sleep into single daily bouts, as do humans and squirrel monkeys, they have multiple sleep episodes interspersed with short wake episodes during their "inactive" period, and vice versa during the "active" period. These active and inactive periods can be organized such that the active period occurs during the nighttime (nocturnal; e.g., rats), daytime (diurnal; e.g., dogs), or at dawn and dusk (crepuscular; e.g., cats). The degree to which animals are polyphasic (i.e., how consolidated is the sleep and wake) can be influenced by locomotor activity. The availability and use of a running wheel in mice causes a decrease in the number of sleep bouts and an increase in the length of wake bouts during the active period (43). This results in a greater amount of wake

during the active period and less fragmented sleep during the inactive period. The hypocretin system may help in this consolidation as hypocretin-1 exhibits a robust increase in response to locomotor activity in polyphasic species, as well as being involved in the control of the timing of wakefulness. Thus, it appears that enhancement of hypocretin-1 signaling promotes wake-consolidation caused by locomotion.

While locomotion can likely have a positive influence on hypocretin tone, locomotion alone is neither necessary nor sufficient to drive hypocretin-1 release. A 50 minute immobilization in rats (41) or a $>80\%$ reduction in locomotor activity for up to 12 hours in squirrel monkeys (40) were unable to significantly affect cisternal CSF hypocretin-1 concentrations. An 8 hour immobilization of rats (41) was able to significantly affect hypocretin-1 concentrations, but this was likely due to a change in sleep/wake behavior (see below). Furthermore, SCN lesioned rats fail to exhibit a diurnal rhythm of cisternal CSF hypocretin-1 when housed in a light/dark cycle, despite the occurrence of a diurnal rhythm in locomotion (caused by positive and negative masking) (25). These data are in sharp contrast to Fos data in cat hypocretin neurons. Fos immunoreactivity in cat hypocretin neurons is elevated after wakefulness with locomotion, but is low after quiet wake (44). This would imply that elevated locomotion is necessary for elevated hypocretin release in cats. This, however, is clearly not the case in rats or squirrel monkeys. It remains to be seen whether the cat hypocretin system is regulated in a fundamentally different manner than is that of the rat or squirrel monkey, or if this is another example of a mismatch between Fos expression in neurons and release patterns of peptides (24). The evidence thus far would suggest that increased locomotion indirectly enhances hypocretin-1 release in polyphasic species, but does not provide a necessary drive to hypocretin neurons.

There is evidence that increases in hypocretin, possibly via the NPY system, can also influence the activity of the hypothalamic-pituitary-adrenal (HPA) axis, the main circuit responsible for immediate responses to stress. As many of the other behaviors influenced by hypocretin can feedback onto the hypocretin system, it is possible that stress could do so as well. There is, however, scant evidence that cortisol or corticosterone (the main outputs of the HPA axis in primates and rodents, respectively) can feedback onto and affect hypocretin neurons. In SCN lesioned rats kept in constant darkness, there was no correlation between cisternal CSF concentrations of cortisol and hypocretin-1 ($r = 0.1$, $p = 0.52$) (25). The correlation between cortisol and hypocretin-1 has also been examined in squirrel monkeys under activated conditions (Figure 3). As described above, while CSF hypocretin was unchanged during a short (<12 hour) food deprivation, there was a robust increase in CSF cortisol (avg $\pm$ sem: $144\% \pm 38\%$; $p < 0.05$, paired T-test) (Fig. 3 top panel; Zeitzer et al., unpublished observations). Also described above, an average ($\pm$sem) reduction in motor activity of $75.3\% \pm 6.65\%$ from 07:00 until 13:00 had no significant effect on CSF hypocretin-1 concentrations. This occurred despite a $165\% \pm 15.4\%$ (avg $\pm$ sem) increase in CSF cortisol concentrations (Fig. 3, bottom panel). Even under circumstances, such as sleep deprivation, when both CSF hypocretin-1 and cortisol increase (45), there is no correlation between changes in the two ($r = 0.29$, $p = 0.44$). Further, in this sleep deprivation protocol there was an increase in cortisol in 9 out of 10 monkeys and an increase in hypocretin-1 in 9 out of 10 monkeys, but the one monkey that failed to exhibit an increase in cortisol had a normal increase in hypocretin and the one monkey that failed to exhibit an increase hypocretin-1 had a normal increase

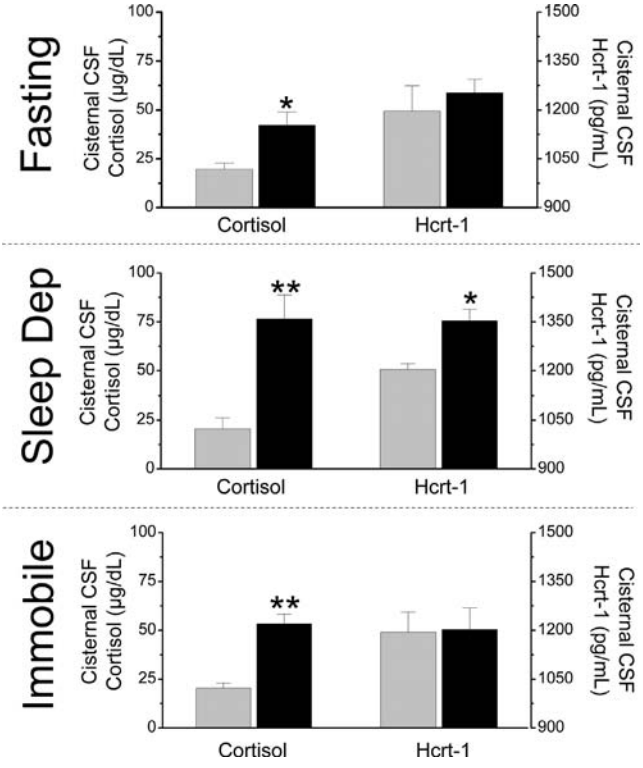


Figure 3 Changes in cortisol and hypocretin-1 are not necessarily correlated in the squirrel monkey. We have observed three conditions in which cisternal CSF cortisol has a robust increase in the squirrel monkey: fasting, sleep deprivation, and immobilization (cortisol is the main "stress-evoked" output of the HPA axis in squirrel monkeys). While cortisol increased in the immobilization and fasting paradigms (cf., *grey bars*, time-matched baseline; *black bars*, intervention), there was no significant change in hypocretin-1 in either protocol. The sleep deprivation paradigm caused an increase in both cortisol and hypocretin, but the changes were not correlated with one another. There appears no functional or necessary correlation between evoked cortisol and changes in hypocretin-1. *, $p < 0.05$; **, $p < 0.01$.

in cortisol. These results indicate that there is no necessary relationship between changes in cortisol and changes in hypocretin-1, and that there is little if any feedback of cortisol on the squirrel monkey hypocretin system. More data are needed to understand if there is physiologically significant feedback of corticosterone on the rodent hypocretin system, as would be expected, as increases in corticosterone release would likely indicate situations in which wakefulness would be beneficial for the organism.

VII. Activation of Hypocretin Release by Sleep Deprivation

As the main function of the hypocretin system is to regulate the occurrence of the wake state, it is also important to determine if the mere presence of wakefulness can feedback and influence the hypocretin system. Hypocretin neurons can be modulated

by the neurotransmitters involved in wake-state control: acetylcholine, noradrenaline, serotonin, and dopamine (46,47). This indicates that the cholinergic, noradrenergic, serotonergic and dopaminergic nuclei to which hypocretin neurons project may provide reciprocal feedback to stabilize the wake promoting function of hypocretin neurons. Interestingly, while hypocretin provides a significant innervation to the wake-promoting TMN histaminergic neurons, hypocretin neurons are not sensitive to *in vitro* histamine application (46). Changes in sleep behavior have been shown to influence cisternal CSF concentrations of hypocretin-1. In rats (39), dogs (35), and monkeys (45), sleep deprivation increases hypocretin-1 concentrations. A minimum amount of sleep deprivation is necessary to induce changes in hypocretin-1 release. Changes are observed in rat hypothalamic microdialysate concentrations of hypocretin-1 after a minimum of 6 hours of sleep deprivation, with no changes being observed after up to 4 hours of sleep deprivation (39). Similarly in squirrel monkeys, we have also observed that while 2 hours of sleep deprivation has no effect on cisternal CSF concentrations of hypocretin-1, 4 or more hours of sleep deprivation will cause a consistent, significant increase in concentrations (Zeitzer et al., unpublished data and ref. 45). It is interesting to note that sleep deprivation increases hypocretin-1 in all species thus far studied, as opposed to other behaviors that affect the hypocretin system of polyphasic species to a much greater degree than the hypocretin system in species that consolidate wakefulness. This may indicate that either an increased drive on hypocretin due to a longer time awake or the lack of negative drive on hypocretin due to the prohibition of sleep (or a combination of these two factors) is critical to the regulation of the hypocretin system of humans. Future experimentation needs to address the responsiveness of the human hypocretin system to sleep deprivation, as well as the neurologic or humoral substrate of this feedback mechanism.

VIII. Sleep Stage Regulation of Hypocretin Activity

It is not known if the hypocretin system is involved in the regulation of transitions or the occurrence of the various states of sleep. The fact that narcolepsy caused by hypocretin deficiency has clear state dysregulation, however, suggests such a role. Cisternal CSF concentrations of hypocretin-1 in the rat peak just before the onset of sleep and decline progressively during the inactive period, reaching nadir before typical wake time (Fig. 2). This pattern is also true in the squirrel monkey which, as is the case with humans, has a predominance of REM sleep at the end of the night. This would imply that a decrease in the release of hypocretin would be either permissive or causal for the occurrence of REM sleep. This is supported by recent *in vivo* electrophysiologic data in rats (48). Single unit recordings from *post-hoc* identified hypocretin neurons were done in freely behaving (head-fixed) rats. Elevated firing was observed during wakefulness, with especially robust firing during wakefulness associated with movement. Low or absent firing was associated with both slow wave sleep (SWS) and REM sleep. An increase in firing of hypocretin neurons occurred just prior to transitions to wakefulness. These data indicate that hypocretin neurons may be less important for transition between various states of sleep and may be more involved in the generation of wakefulness such their quiescence may be necessary for the occurrence of sleep. These data are, however, preliminary and replication will be needed.

Data from cats are, however, contrary to those obtained by electrophysiology in rats. In cats, microdialysate concentrations of hypocretin-1 are elevated during REM sleep, as compared with SWS, in the hypothalamus and basal forebrain, though not the locus coeruleus (49). It must be noted, though, that in these data, hypocretin-1 release during SWS was not different from that during quiet wakefulness, in any of the probes. Given the brevity of REM episodes in cats and the poor temporal resolution of microdialysis (compared with electrophysiology), more data are necessary to determine if regulation of hypocretin neurons in the cat is fundamentally different from their regulation in rats and, likely, humans and squirrel monkeys.

IX. Functional Relevance to the Narcolepsy Phenotype

If we presume that there is a general increase in hypocretin tone late in the waking period, the question arises as to the functional significance of this pattern. A clue to the physiologic role of the hypocretin system in human wake physiology came from a study by Dantz and colleagues (16). Dantz *et al.* studied the electroencephalograph (EEG) activity of narcoleptic and control subjects during a 90-minute forced desynchrony protocol (60 minutes of scheduled wake followed by 30 minutes of scheduled sleep in darkness, in every 90 minutes for 2–3 days). In such a protocol, the circadian and homeostatic components of sleep and wake regulation can be separated. Sleep and wake in humans is postulated to be regulated by two processes, a circadian clock and a homeostatic drive (50). The former is a daily signal that occurs with a 24-hour periodicity, irrespective of sleep and wake state. The latter is an appetitive process, in which the longer the time spent awake, the greater the drive for sleep, and the longer the time spent asleep, the less the drive for sleep. Dantz found that narcoleptics had a normal homeostatic response to sleep loss, but they failed to exhibit an increase in wakefulness during the late afternoon. A study of ambulatory narcoleptic subjects substantiated this finding as it showed that individuals with narcolepsy were readily able to sleep during the late afternoon, a time at which napping is rarely observed in non-narcoleptic individuals (17). This increased wakefulness in the later afternoon is mathematically modeled to represent a circadian-driven alertness signal. It appears, therefore, that individuals with narcolepsy have a normal sleep/wake homeostat, but a dysfunctional circadian alertness signal (though otherwise normal circadian timing system). Under entrained conditions, this burst of alertness during the late afternoon/early evening is the drive that allows humans to stay awake continuously for 16 hours. As narcoleptics with cataplexy mostly have a deficient hypocretin system (51), it is likely that *hypocretin is specifically involved in the expression of this circadian alertness signal and the wake consolidation process in humans*. As one of the main physiologic functions of hypocretin in humans is to consolidate wake, it is critical to use an animal model in which wake consolidation occurs, such as the squirrel monkey.

The fact that hypocretin transmission is stimulated by sleep deprivation in all species is also likely to be relevant to the phenotype of human narcolepsy. In squirrel monkeys, a sleep deprivation of as little as 4 hours is sufficient to activate the hypocretin system. *It may be that the hypocretin system is critical to maintain consolidated wakefulness in the face of a mild sleep debt.* This would explain another important feature in narcolepsy, the inability to stay awake for periods longer than a few hours.

Indeed, narcoleptic patients are typically refreshed after sleeping, but the effect usually lasts only a few hours, possibly because this hypocretin activation is not available to counteract the mounting sleep debt.

Another symptom of narcolepsy that deserves discussion in the context of this chapter is the occurrence of abnormal, dissociated REM sleep events such as short REM latency, hypnagogic hallucinations, and sleep paralysis. Each of these may be explained by the removal of hypocretin's excitatory effects on monoaminergic and other systems. As mentioned above, it is likely that hypocretin neuron firing is low during REM sleep, in agreement with this hypothesis (i.e., the constitutively absent hypocretin tone is permissive for REM-related events at any time in narcoleptics). The picture may, however, be more complex with regards to cataplexy. CSF hypocretin-1 concentrations are abnormally low in $\sim$90% of narcoleptics with cataplexy, but are low in only 7-30% of those without cataplexy. Cataplexy is a fairly unique symptom that may not be simply related to abnormal REM sleep. REM sleep or total sleep deprivation, for example, is not known to induce this symptom, whereas the occurrence of other abnormal REM sleep events (e.g., sleep paralysis, hypnagogic hallucinations) may increase during sleep deprivation in non-narcoleptic individuals. The occurrence of cataplexy may, thus, be due to the impairment of very specific, probably unknown, hypocretin projections. The fact that hcrtr2 mutated animals have cataplexy suggests these putative projections are likely to involve this excitatory receptor. It has recently been proposed that impaired projections to the hcrtr1 enriched locus coeruleus may be responsible for cataplexy (52). This is, we believe, unlikely as hcrtr1 mutated rodents, unlike hcrtr2 mutated animals, do not have cataplexy (53). Similarly, dopamine beta-hydroxylase knockout animals, which do not have norepinephrine, have a mild sleep phenotype but exhibit no cataplexy. We, therefore, rather believe that adrenergic transmission is an important, independent modulator of muscle tone and cataplexy that is not by itself involved in the generation of cataplexy.

Finally, two other common symptoms in narcolepsy deserve some discussion: obesity and disturbed nocturnal sleep. As mentioned above, hypocretin activity and metabolism are coupled, at least in some species. Obesity has been reported in human narcolepsy but it is a variable symptom that affects only a subset of patients. As first reported by Daniels in 1934 (54), we believe the increased incidence of obesity in narcoleptics is most clear when disease onset is abrupt and patients are not aggressively treated close to onset. Most probably, it is the result of an abrupt increase in inactivity in some patients as the result of sleepiness. A survey of over 400 treated narcoleptic patients indicated a body mass index only 10% greater than controls, suggesting obesity may resolve if patients are adequately treated (55).

The occurrence of disturbed nocturnal sleep is a more consistent finding, affecting approximately half of all narcoleptics. It generally occurs later in the course of the disease. Importantly, however, even when it is not a major complaint, sleep is usually disturbed when examined with polysomnography, suggesting a genuine abnormality. Furthermore, pharmacological consolidation of wake generally does not improve sleep disruption, suggesting a primary abnormality. The occurrence of sleep disruption in narcolepsy has been best discussed in the context of the flip-flop model of sleep-wake regulation (56). In this elegant model, wake- and sleep-promoting neurons are proposed to inhibit one another to increase the stability of each state. Hypocretin, with its projections to monoaminergic nuclei, would directly excite the wake-promoting areas of the

brain. Hypocretin would also presynaptically increase the inhibitory monoaminergic influence on the sleep-promoting preoptic area. Hence, in this model, the absence of hypocretin would lead to a destabilization of sleep and wake consolidation, which is observed in narcolepsy. An additional factor explaining sleep disruption may also be the preferential disinhibition of REM sleep.

X. Perspectives

In humans and other species in which sleep and wake are consolidated into single daily episodes, the hypocretin system is likely involved in the expression of the circadian alertness signal, in maintaining wakefulness in the face of a sleep debt, and in the inhibition of REM sleep. In polyphasic species (e.g., mice, rats, dogs, cats), the hypocretin system is also likely controlled by the circadian clock, but the late day increase in hypocretin-1 release is unable to consolidate wakefulness into a single bout. However, the hypocretin system in polyphasic species appears acutely sensitive to the concomitants of wakefulness (e.g., locomotion, stress, feeding). When a polyphasic animal is moving about, stressed, feeding, and so on, hypocretin-1 release is enhanced and wake consolidation is increased. These behaviors have little effect on the hypocretin system of the squirrel monkey, a species, like humans, that consolidates wake into a single daily episode without a necessary stimulation from the external environment.

It would appear, then, that the main function of hypocretin is to consolidate the behaviors of wakefulness into a single daily episode and that this function occurs without stimulation in monophasic species (human, squirrel monkey), but requires additional stimulation from the concomitants of wakefulness in polyphasic species. The neural or humoral substrate of this feedback in polyphasic animals will be critical to determine as it may aid in the treatment of individuals who have a dysfunctional hypocretin system (narcolepsy).

XI. Summary

That the hypocretin (orexin) system has a role in regulating sleep and alertness in humans is incontrovertible – humans who lack normal production of this peptide have symptoms of narcolepsy (hypersomnia, disrupted nocturnal sleep, possible intrusion of rapid eye movement (REM) sleep-related events into wakefulness). Examination of the anatomy and physiology of the hypocretin system supports this role and has, further, shed light on the specific function that the hypocretin system may have in the regulation of normal sleep and alertness.

References

1. de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proceedings of the National Academy of Sciences USA 1998; 95:322–327.
2. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and g protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.

3. van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. Journal of Neuroscience 1999; 19(8):3171–3182.
4. Peyron C, Tighe DK, van den Pol A, de Lecea L, Heller HC, Sutcliffe JG et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. Journal of Neuroscience 1998; 18(23): 9996–10015.
5. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proceedings of the National Academy of Sciences USA 1999; 96:748–753.
6. Trivedi P, Yu H, MacNeil DJ, van der Ploeg LHT, Guan X-M. Distribution of orexin receptor mRNA in the rat brain. FEBS Letters 1998; 438:71–75.
7. Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M et al. Differential expression of orexin receptors 1 and 2 in the rat brain. Journal of Comparative Neurology 2001; 435:6–25.
8. Burdakov D, Liss B, Ashcroft FM. Orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium-calcium exchanger. Journal of Neuroscience 2003; 23(12):4951–4957.
9. Hoang QV, Bajic D, Yanagisawa M, Nakajima S, Nakajima Y. Effects of orexin (hypocretin) on GIRK channels. Journal of Neurophysiology 2003; 90(693):702.
10. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY et al. Distinct narcolepsy syndromes in *orexin receptor-2* and *orexin* null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. Neuron 2003; 38:715–730.
11. Ida T, Nakahara K, Katayama T, Murakami N, Nakazato M. Effect of lateral cerebroventricular injection of the appetite-stimulating neuropeptide, orexin and neuropeptide Y, on the various behavioral activities of rats. Brain Research 1999; 821:526–529.
12. Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proceedings of the National Academy of Sciences USA 1999; 96:10911–10916.
13. Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M et al. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. Brain Research 2000; 873: 181–187.
14. Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T. Orexin-A regulates body temperature in coordination with arousal status. Experimental Biology and Medicine 2001; 226(5):468–476.
15. España RA, Plahn S, Berridge CW. Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. Brain Research 2002; 943:224–236.
16. Dantz B, Edgar DM, Dement WC. Circadian rhythms in narcolepsy: studies on a 90 minute day. Electroencephalography and Clinical Neurophysiology 1994; 90:24–35.
17. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Research Online 1998; 1(4):159–165.
18. Bourgin P, Huitrón-Reséndiz S, Spier AD, Fabre V, Morte B, Criado JR et al. Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. Journal of Neuroscience 2000; 20(20):7760–7765.
19. Xi M-C, Morales FR, Chase MH. Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. Brain Research 2001; 901:259–264.
20. Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan J, Wang Q-P et al. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochemical and Biophysical Research Communications 2002; 290:1237–1245.
21. Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. Journal of Neuroscience 2002; 21(23):9273–9279.
22. Ishizuka T, Yamamoto Y, Yamatodani A. The effect of orexin-A and -B on the histamine release in the anterior hypothalamus in rats. Neuroscience Letters 2002; 323:93–96.
23. Huang Z-L, Qu W-M, Li W-D, Mochizuki T, Eguchi N, Watanabe T et al. Arousal effect of orexin A depends on activation of the histaminergic system. Proceedings of the National Academy of Sciences USA 2001; 98(17):9965–9970.
24. Hoffman GE, Lyo D. Anatomical markers of activity in neuroendocrine systems: are we all 'fos-ed out'? Journal of Neuroendocrinology 2002; 14:259–268.
25. Zhang S, Zeitzer JM, Yoshida Y, Wisor JP, Nishino S, Edgar DM et al. Lesions of the suprachiasmatic nucleus eliminate the daily rhythm of hypocretin-1 release. Sleep 2004; 27(4):619–627.

26. Deboer T, Overeem S, Visser NA, Duindam H, Frolich M, Lammers G et al. Hypocretin-1 is under influence of circadian and homeostatic mechanisms. Sleep 2004; 27:A1-A2.
27. Abrahamson EE, Leak RK, Moore RY. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. NeuroReport 2001; 12(2):435–440.
28. Chou TC, Scammell TE, Gooley JJ, Gaus SE, Saper CB, Lu J. Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. Journal of Neuroscience 2003; 23(33): 10691–10702.
29. Di Chiro G, Larson SM, Harrington T, Johnston GS, Green MV, Swann SJ. Descent of cerebrospinal fluid to spinal subarachnoid space. Acta Radiologica (Diagnosis) 1973; 14:379–384.
30. Abrahamson EA, Moore RY. The posterior hypothalamic area: chemoarchitecture and afferent connections. Brain Research 2001; 889:1–22.
31. Campbell RE, Smith MS, Allen SE, Grayson BE, ffrench-Mullen JMH, Grove KL. Orexin neurons express a functional pancreatic polypeptide Y4 receptor. Journal of Neuroscience 2003; 23(4):1487–1497.
32. Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J et al. Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. Journal of Comparative Neurology 1998; 402:442–459.
33. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 2003; 38:701–713.
34. Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S. Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 hours and in response to food deprivation. NeuroReport 2001; 12(5):993–997.
35. Wu M-F, John J, Maidment N, Lam HA, Siegel JM. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. American Journal of Physiology 2002; 283:R1079-R1086.
36. Salomon RM, Ripley B, Kennedy JS, Johnson B, Zeitzer JM, Nishino S et al. Diurnal variation of CSF hypocretin-1 (orexin-A) levels in control and depressed subjects. Biological Psychiatry 2003; 54:96–104.
37. Blank JL, Desjardins C. Differential effects of food restriction on pituitary-testicular function in mice. American Journal of Physiology 1985; 248:R181-R189.
38. Bae HH, Larkin JE, Zucker I. Juvenile Siberian hamsters display torpor and modified locomotor activity and body temperature rhythms in response to reduced food availability. Physiological and Biochemical Zoology 2003; 76(6):858–867.
39. Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H, Yoneda H et al. Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. European Journal of Neuroscience 2001; 14:1075–1081.
40. Zeitzer JM, Buckmaster CL, Lyons DM, Mignot E. Locomotor-dependent and -independent components to hypocretin-1 (orexin A) regulation in sleep-wake consolidating monkeys. Journal of Physiology 2004; 557(3):1045–1053.
41. Martins PJF, D'Almeida V, Pedrazzoli M, Lin L, Mignot E, Tufik S. Increased hypocretin-1 (orexin-a) levels in cerebrospinal fluid of rats after short-term forced activity. Regulatory Peptides 2004; 117: 155–158.
42. Torterolo PD, Sampogna S, Ramos O, Morales FR, Chase MH. Fos immunoreactivity in hypocretinergic and MCHergic neurons in the cat during different somatomotor behaviors. Society for Neuroscience 34, 437.16. 2004.
43. Welsh DK, Richardson GS, Dement WC. Effect of running wheel availability on circadian patterns of sleep and wakefulness in mice. Physiology and Behavior 1988; 43:771–777.
44. Torterolo P, Yamuy J, Sampogna S, Morales FR, Chase MH. Hypothalamic neurons that contain hypocretin (orexin) express *c-fos* during active wakefulness and carbachol-induced active sleep. Sleep Research Online 2001; 4(1):25–32.
45. Zeitzer JM, Buckmaster CL, Parker KJ, Hauck CM, Lyons DM, Mignot E. Circadian and homeostatic regulation of hypocretin in a primate model: implications for the consolidation of wakefulness. Journal of Neuroscience 2003; 23(8):3555–3560.
46. Yamanaka A, Muraki Y, Tsujino N, Goto K, Sakurai T. Regulation of orexin neurons by the monoaminergic and cholinergic systems. Biochemical and Biophysical Research Communications 2003; 303:120–129.
47. Li Y, Gao X-B, Sakurai T, van den Pol AN. Hypocretin/orexin excites hypocretin neurons via a local glutamate neuron - a potential mechanism for orchestrating the hypothalamic arousal system. Neuron 2002; 36:1169–1181.

48. Lee M, Jones BE. Discharge of identified orexin neurons across the sleep-waking cycle. Society for Neuroscience 34, 841.1. 2004.
49. Kiyashchenko LI, Mileykovskiy BY, Maidment NT, Lam HA, Wu M-F, John J et al. Release of hypocretin (orexin) during waking and sleep states. Journal of Neuroscience 2002; 22(13):5282–5286.
50. Dijk D-J, Czeisler CA. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neuroscience Letters 1994; 166:63–68.
51. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Archives of Neurology 2002; 59:1553–1562.
52. John J, Wu M-F, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron 2004; 42(4):619–634.
53. Kisanuki YY, Chemelli RM, Sinton CM, Williams SC, Richardson JA, Hammer RE et al. The role of orexin receptor type-1 (OX1R) in the regulation of sleep. Sleep 23, A91. 2000.
54. Daniels LE. Narcolepsy. Medicine (Baltimore) 1934; 13:121–122.
55. Okun ML, Lin L, Pelin Z, Hong S, Mignot E. Clinical aspects of narcolepsy-cataplexy across ethnic groups. Sleep 2002; 25(1):27–35.
56. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends in Neurosciences 2001; 24(12):726–731.
57. Taheri S, Sunter D, Dakin C, Moyes S, Seal L, Gardiner J et al. Diurnal variation in orexin A immunoreactivity and prepro-orexin mRNA in the rat central nervous system. Neuroscience Letters 2000; 279:109–112.
58. Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M et al. Fos expression in orexin neurons varies with behavioral state. Journal of Neuroscience 2001; 21(5):1656–1662.

34
Hypocretin, $GABA_B$ Receptors and Sleep

THOMAS S. KILDUFF, STEPHEN R. MORAIRTY, TERESA L. STEININGER, and XINMIN S. XIE
Biosciences Division, SRI International, Menlo Park, California, U.S.A.

I. Introduction

The discovery of the hypocretins [Hcrt; (1)] [also known as the orexins (2)], the receptors for these peptides (2) and the subsequent links of defects in this neuropeptide system to narcolepsy or narcoleptic-like syndromes in dogs (3), mice (4) and humans (5,6) has raised the prospect of new therapeutic avenues for narcoleptic patients. One of the earliest neuroanatomical studies of the Hcrt system indicated that the axonal projections of these cells terminated in brain regions that have been classically implicated in arousal state control (7). These anatomical relations of the Hcrt cells, coupled with pathology of wakefulness that results from disruption of the Hcrt system, led us to propose a model in which the Hcrt neurons played a central role in the control of behavioral state (8). A key component of this model was a putative role for inhibitory GABAergic input to the Hcrt cells in mediating the transition from wakefulness to slow wave sleep. This feature of our model has been retained in other recently proposed models (9,10).

In contrast to the efferent connections of Hcrt cells, the afferent innervation of Hcrt neurons is not well understood. Although a complete summary of the afferent connections of the Hcrt cells is beyond the scope of this chapter, we have recently summarized the literature published through June, 2004 (11). At the time we proposed the model, there was no direct evidence for GABA input to the Hcrt cells per se but our thinking was influenced by the evidence for increased GABA release in the posterior hypothalamus during SWS (12). Since then, in vitro electrophysiological studies have established that the Hcrt cells are indeed inhibited in response to GABA application (13–15). The primary focus to date has been on GABAergic modulation of Hcrt cells through $GABA_A$ receptors (13,14) and anatomical studies have established the presence of the $GABA_A$ receptor epsilon subunit on Hcrt neurons (16). However, $GABA_B$ receptor mRNA has recently been shown to be present in Hcrt neurons (17) and we have recently obtained evidence of modulation of Hcrt cells through $GABA_B$ receptors as well (15). As described below, this $GABA_B$-mediated mechanism may be related to the therapeutic activity of gamma-hydroxybutyrate, a current treatment for narcolepsy.

II. Narcolepsy, Gamma-Hydroxybutyrate, and the Hypocretin System

A. Gamma-Hydroxybutyrate as a Therapeutic for Narcolepsy

Gamma-hydroxybutyrate (GHB) is a hypnotic compound that is noteworthy for its ability to induce physiologically normal slow wave sleep (18) in contrast to the benzodiazepines, which are well known to suppress slow wave sleep even though they decrease sleep latency and sleep fragmentation (19). GHB is produced endogenously as a metabolite of the inhibitory neurotransmitter GABA. A pharmaceutical formulation of GHB known as Xyrem® was approved for treatment of the cataplexy component of narcolepsy in 2002 (20). GHB is an effective anticataplectic therapeutic but is atypical in some respects. Unlike most narcolepsy therapeutics, GHB effectively treats both excessive daytime sleepiness (EDS) and cataplexy when administered at bedtime rather than during the day. Furthermore, its effects emerge after several days of GHB treatment, rather than as an immediate consequence of administration, and the suppression of cataplexy and EDS are sustained for several days subsequent to GHB withdrawal. The indirect and sustained efficacy of GHB in treating cataplexy and EDS in narcolepsy likely reflects profound changes in gene expression or sleep-related neural pathways. However, the neurochemical and physiological changes that underlie these sustained effects of repeated GHB administration are incompletely understood (21).

Like its chemical relative GABA, GHB activates $GABA_B$ receptors (22), and many of the physiological effects of GHB are abolished in mice lacking $GABA_B$ receptors (23,24). However, a putative GHB receptor was recently cloned (25). The specificity of GHB for this receptor and the fact that it is an agonist and not an allosteric modulator may explain some of the differences between the physiological responses to GHB and benzodiazepines. Since the mechanism underlying the hypnotic action of GHB remains controversial, we have undertaken a series of studies to explore the neural substrates of the effects of GHB. Our approach to date has involved a combination of in vivo recording of sleep/wake states, functional neuroanatomy, and in vitro patch clamp electrophysiological studies.

B. In Vivo Studies with Gamma-Hydroxybutyrate

In the first study, male Sprague-Dawley rats ($n = 4$) were prepared with chronic electrode implants for continuous EEG and EMG recordings. EEG electrodes were positioned bilaterally at +2.0 mm AP from bregma and 2.0 mm ML and at −6.0 mm AP and 3.0 mm ML. Animals were housed in a temperature controlled recording room under a 12/12 light/dark cycle and had food and water available *ad libitum*. Room temperature, humidity and lighting conditions were monitored continuously via computer. After a recovery period of 7 to 10 days, rats were connected via a cable and a counter-balanced commutator to a digital data collection system (Somnologica, MedCare; Reykjavik, Iceland) for EEG and EMG recording. The animals were allowed to acclimate to the recording environment at least 48 hours before the injection. Approximately 3 hours following lights onset (ZT3) on the experimental days, either sterile saline or Xyrem® was administered intraperitoneally (i.p.) at 300, 600, or 800 mg/kg in volumes ranging from 0.2 to 0.6 mL. Doses were randomized and counterbalanced between animals; a minimum of two days elapsed between dosings for any

individual animal. EEG and EMG signals were amplified and digitized using Somnologica and stored on a computer; these data were scored visually in 10 seconds epochs as waking (W), rapid eye movement sleep (REMS) or nonrapid eye movement sleep (NREMS) by expert scorers using SleepSign (Kissei Comtec, Irvine, California). Scored data were analyzed and expressed as time spent in each state per hour. Sleep bout duration and bout frequency were analyzed as well.

At the two highest doses used, animals became immobile within 2–5 minutes after treatment. Spike and wave discharge was evident in the EEG, as described by others (26). Figure 1a shows EEG and EMG activity recorded from a rat before treatment (upper two traces), during the first hour after treatment with 800 mg/kg of Xyrem® (middle two traces), and 4 hours after Xyrem® treatment (bottom two traces). At the two highest doses used, the spike and wave discharge evident in the middle two traces in Figure 1a persisted as long as 3 hours after injection but spike

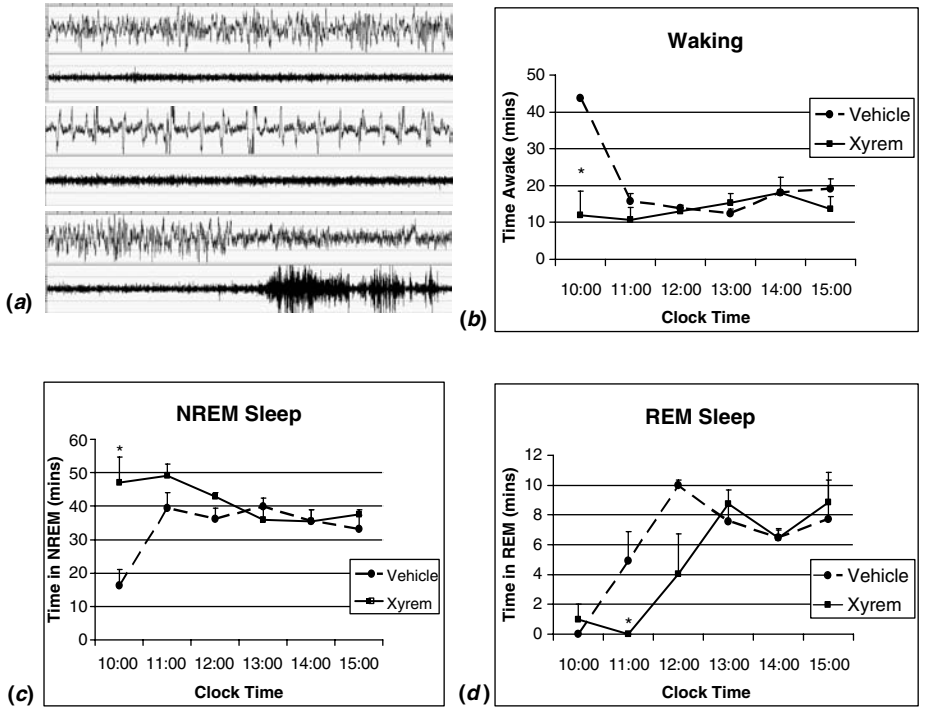


Figure 1 The effects of Xyrem on behavioral state in rats. (***a***) The effects of a high dose of Xyrem^R (800 mg/kg i.p.) on the EEG and EMG from an individual rat. The top two traces are baseline EEG and EMG, respectively, approximately one hour prior to Xyrem^R administration. The middle two traces were captured during the first hour following Xyrem^R administration and show spike and wave seizure activity induced by high concentrations of Xyrem^R. The bottom two traces show recovery of the EEG and EMG to baseline levels 4 h post- Xyrem^R. (***b–d***) Effects of 300 mg/kg i.p. administration of Xyrem^R on waking, NREM and REM sleep for 6 h following injection during the light period (lights on at 0700). administration. *, $p < 0.05$.

and wave activity was never observed at the 300 mg/kg dose. Spike and wave activity is an abnormal EEG pattern similar to that seen in the human EEG during certain types of epilepsy, as well as in animal models of absence seizures. Consequently, we used 300 mg/kg as the dose for subsequent studies.

Figures 1b–1d illustrate the effects of Xyrem® (300 mg/kg, i.p.) on wakefulness, NREMS and REMS for 6 hours after injection. A profound decrease ($p < 0.05$) in wakefulness (Figure 1B) and increase in NREMS (Fig. 1c) occurred during the first hour after injection. NREMS remained at high levels during postinjection hour 2 but recovered to near baseline levels by the third hour. REMS (Fig. 1d) was significantly ($p < 0.05$) suppressed during the second postinjection hour, likely due to the high levels of NREMS which occurred during this time. During postinjection hours 4–6, the vehicle and drug-treated groups were indistinguishable. Based on these results, we can conclude that a 300 mg/kg i.p. dose of Xyrem® in Sprague-Dawley rats transiently increases NREMS while suppressing REMS, without evoking the spike and wave discharge evident at the higher doses.

C. Functional Neuroanatomical Studies with Gamma-Hydroxybutyrate

Our second study involved use of c-Fos immunohistochemistry as a marker of functional activity. This methodology has been widely used throughout neuroscience and, within the sleep field, c-Fos has been used to identify cells activated as a consequence of sleep deprivation (27,28), during REM sleep (29–31) and sleep or wakefulness induced by pharmacological agents (32–34) and anesthetic drugs (35). Based on the existing literature, we expected Xyrem® treatment to decrease the number of Fos-immunoreactive (Fos-IR) neurons in wake-related neuronal populations such as both the Hcrt cells and the histaminergic neurons of the tuberomammillary nucleus (TM), since Fos-IR in these cell groups has been linked to waking activity rather than to time of day (36,37). As a comparison, we also examined Fos-IR in cell populations previously implicated in the control of behavioral state including monoaminergic groups in the brainstem and cholinergic cells groups in both the basal forebrain and pontine tegmentum.

Xyrem® (300 mg/kg i.p.) or sterile saline was administered to Sprague-Dawley rats (300–350 g, Harlan; N = 12) at ZT3. Animals were overdosed with pentobarbital 60 minutes following test injection and transcardially perfused with fixative (4% paraformaldehyde in 0.1 M phosphate buffer). The brains were removed and cryoprotected in sucrose solution. Brain sections (30 μm thickness) were cut on a freezing microtome. Fos immunohistochemistry (IHC) was performed followed by IHC for one of the following neural markers: choline acetyltransferase (ChAT) IHC to visualize cholinergic neurons in the basal forebrain, laterodorsal and pedunculopontine tegmental nuclei; adenosine deaminase IHC to identify histaminergic neurons in the TM; serotonin IHC to localize serotonergic neurons in the dorsal raphe nucleus; tyrosine hydroxylase IHC to mark noradrenergic neurons in the locus coeruleus; tyrosine hydroxylase IHC to label dopaminergic neurons in the ventral tegmental area and the substantia nigra; hypocretin-2 IHC to determine hypocretinergic neurons in the perifornical LHA; and glutamic acid decarboxylase IHC for GABAergic neurons in the preoptic hypothalamus. Fos-positive neurons were identified by the presence of black-precipitate

[Ni^+-enhanced diaminobenzidine (DAB)] in the nucleus, and neural markers were identified by the presence of amber precipitate in the cytoplasm (nonenhanced DAB).

Although analyses are ongoing at the present time, preliminary results presented in Figure 2 do not support the hypothesis being tested: the number of Fos-positive Hcrt neurons appears to be similar between Xyrem®-treated and control animals. These preliminary results, if supported by subsequent quantitation, would suggest that Xyrem® affects behavioral state by a non-Hcrt mechanism. In fact, preliminary results indicate clear increases in the number of Fos-IR neurons in the Xyrem®-treated group in cell populations other than those mentioned above.

D. Cellular Neurophysiological Studies with Gamma-Hydroxybutyrate

In parallel with the functional neuroanatomical studies described above, we sought to determine whether GHB modulates Hcrt neuron activity in cellular electrophysiological

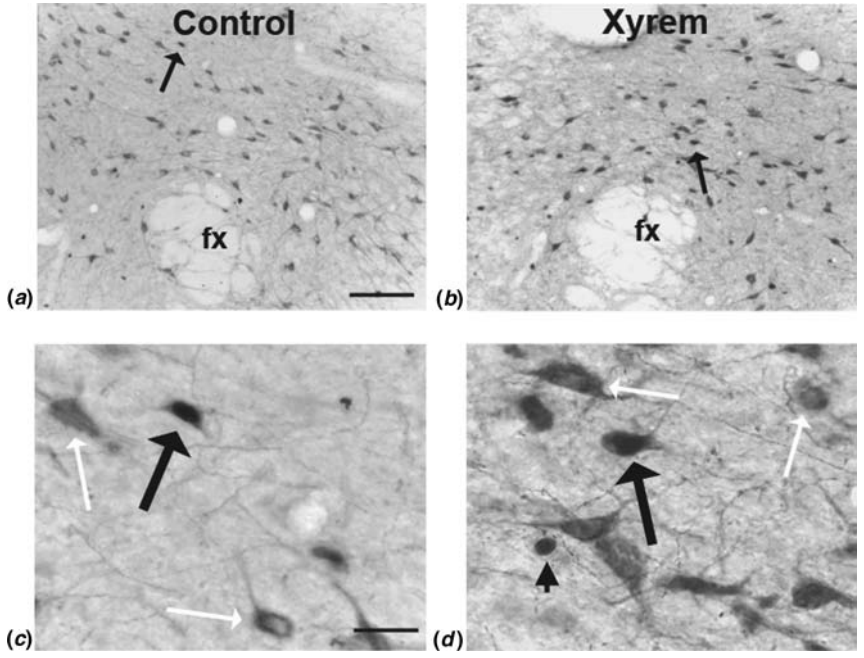


Figure 2 Fos- and Hcrt-immunoreactivity in the perifornical hypothalamus. (***a***) Fos activation in Hcrt neurons following control saline injection. Black arrow indicates Fos/Hcrt double-stained neuron shown at higher magnification in ***c***. (***b***) Fos activation following injection of $Xyrem^R$ (300 mg/kg i.p.). Black arrow indicates Fos/Hcrt double-stained neuron shown at higher magnification in ***d***. (***c***) High power photomicrograph of region shown in ***a***. White arrows indicate Hcrt single-stained neuron, black arrow indicates Fos/Hcrt double-stained neuron. (***d***) High power photomicrograph of region shown in ***b***. Arrows are as indicated in ***c***. Arrowhead indicates Fos single-stained neuron. Scale bar in (***a***) = 200 μm, applies to (***b***); scale bar in (***c***) = 40 μm, applies to (***d***). *Abbreviation*: fx = fornix.

studies. Since the actions of GHB are thought to be mediated in part through $GABA_B$ receptors (22) and because $GABA_B$ receptor mRNA has been shown to be present in Hcrt neurons (17), we evaluated the response of Hcrt neurons to GHB.

To determine whether Hcrt neurons respond directly to GHB application, we conducted in vitro electrophysiological studies in transgenic mice in which enhanced green fluorescent protein (EGFP) was linked to the Hcrt promoter (Hcrt/EGFP), as described previously (13,14). Hypothalamic slice preparation and recording conditions followed the procedures described by Yamanaka et al. (14). Briefly, male and female Hcrt/EGFP mice (3–6 weeks of age) were used for the experiments. Mice were deeply anesthetized with methoxyflurane and then decapitated. Brains were isolated in ice-cold physiological solution bubbled with 95% O_2, 5% CO_2 containing (mM): NaCl 135, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, $NaHCO_3$ 25, glucose 10. Brains were cut coronally into 250-μm slices with a microtome (VT-1000S, Leica, Germany). Slices containing the lateral hypothalamic area were transferred to an incubation chamber where they were superfused with oxygenated physiological solution. Hypothalamic slices were incubated at room temperature (24–26°C) for at least one hour before recording. Hcrt/EGFP neurons were visualized on an upright microscope (Leica DM LFSA, Leica Instruments) using both infrared-differential interference contrast (IR-DIC) microscopy and fluorescence microscopy. Infrared images were acquired via a charge-coupled device (CCD) camera optimized for infrared wavelengths (DAGE-MITI, Michigan City, IN); fluorescent images were acquired using a digitizer system (Leica LEI-750D, Leica Instruments).

Patch pipettes were prepared from borosilicate capillary glass (GC150-10, Harvard Apparatus, Holliston, MA) with a micropipette puller (P-97, Sutter Instruments, Novato, CA). The pipettes were filled with an internal solution containing (mM): KCl 145, $MgCl_2$ 1, EGTA-Na_3 1.1, HEPES 10, Na_2ATP 2, Na_2GTP 0.5, pH 7.2 with KOH. Osmolarity of the solution was checked by a vapor pressure osmometer (Advanced Instruments, Norwood, MA). Pipette resistance was 4–10 Mohms. The series resistance during recording was 10–25 Mohms. Recording pipettes were advanced towards individual fluorescent cells in a slice while under positive pressure and, on contact, tight seals on the order of 0.5–1.0 Gohms were made by negative pressure. The membrane patch was then ruptured by suction and membrane potential was monitored using an Axopatch 1D patch clamp amplifier (Axon Instruments, Foster City, California, U.S.A.).

The basic electrophysiological properties of the Hcrt/EGFP neurons were comparable to those described previously (13,14). Under control conditions, all Hcrt/EGFP neurons recorded ($n = 45$) displayed spontaneous firing of action potentials (0.5–3 Hz) superimposed on spontaneous synaptic activity. The resting potential recorded in these cells was approximately −50 mV; spike overshoots were usually greater than 40 mV. Tetrodotoxin (TTX, 1 μM) blocked spiking and depressed synaptic activity, indicating that the spikes were generated by the activation of TTX-sensitive voltage-gated Na^+ channels and the activity was principally driven by neurotransmission. Bath application of the selective $GABA_B$ agonist (R)-baclofen (10–100 μM) caused hyperpolarization (3–10 mV) and decreases in input resistance (10–35%) and membrane excitability. Figure 3a illustrates the response to application of 10 μM baclofen and to GABA recorded with high KCl as the internal pipette solution to distinguish between $GABA_A$- and $GABA_B$-mediated signaling. GABA (100 μM) produced membrane

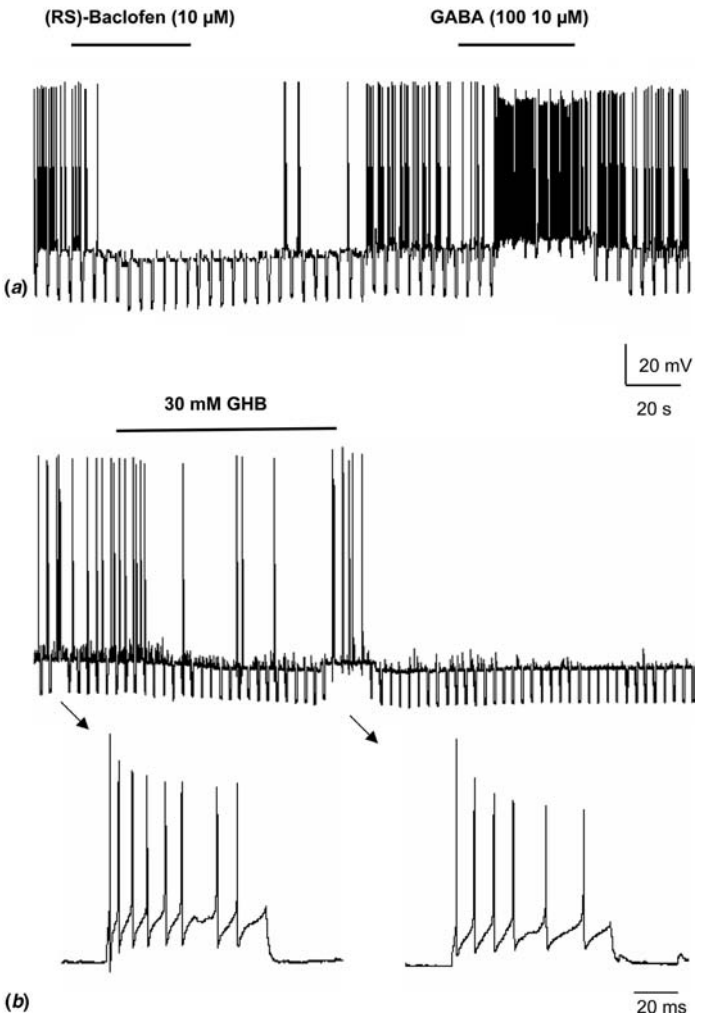


Figure 3 Effects of Xyrem® (GHB), (RS)-baclofen and GABA on membrane potential and firing rate of Hcrt neurons in mouse hypothalamic slices. (***a***) Bath application of (RS)-baclofen (10 μM) caused membrane hyperpolarization (∼5 mV) and blocked spontaneous firing action potentials. In contrast, GABA (100 μM) produced membrane depolarization (∼3 mV), subsequently increased firing of action potentials and dramatically decreased input resistance (from 120 MΩ reduced to 25 MΩ) under our experimental conditions using an internal pipette solution containing 130 mM KCl. (***b***) Xyrem® (GHB, 30 mM) caused membrane hyperpolarization (∼5 mV) and decreased spontaneous firing of action potentials in another Hcrt neuron. In the lower panel, time-expanded voltage record traces taken from where arrows indicate. A train of action potentials was elicited by a depolarizing current injection (50 pA, 800 ms) in the presence and absence of Xyrem®. Hyperpolarizing current pulses (−0.2nA, 800 ms) were delivered every 5 seconds throughout the experiment. Both neurons were held at −60 mV with -DC injection.

depolarization, a subsequent increase in the firing of action potentials, and dramatically decreased input resistance. In the same Hcrt neuron, application of 10 mM Xyrem®, evoked hyperpolarization and a decrease in input resistance (Fig. 3b), consistent with a $GABA_B$-mediated mechanism. These $GABA_B$-mediated responses persisted in the presence of tetrodotoxin (0.5 μM), suggesting direct postsynaptic effects, presumably through activation of the G protein-coupled inwardly rectifying K^+ channels.

These results demonstrate that functional $GABA_A$ and $GABA_B$ receptors exist in Hcrt/EGFP neurons and that GABA can act on both receptors in these cells. The baclofen and GHB-induced responses in Hcrt neurons are consistent with $GABA_B$-mediated responses in other types of neurons, for example, hippocampal pyramidal cells (22). Exogenous GHB at high concentrations selectively activates $GABA_B$ receptors and produces a profound inhibitory effect on the wake-promoting Hcrt neurons. The cellular action of GHB may underlie its in vivo effect of promoting slow wave sleep by inhibition of the wake-promoting Hcrt cells and may contribute to the efficacy of Xyrem® in the treatment of narcolepsy.

III. Perspective and Future Directions

Whereas the functional neuroanatomical studies indicate that GHB does not activate Hcrt neurons, the cellular neurophysiological results clearly demonstrate that GHB can inhibit the activity of these cells. Given these results, an important consideration is the source of GABAergic input. Our model (8) proposed input from the ventrolateral preoptic area (VLPO) as one potential source of GABA. Two recent studies have shown increased c-Fos expression in Hcrt neurons after application of the $GABA_A$ agonist muscimol when microinjected into either the nucleus accumbens shell (38) or the preoptic area (39), implicating these brain regions as sources of GABAergic inhibitory input to the Hcrt cells.

Reduced levels Hcrt-1 in cerebrospinal fluid of narcoleptic patients (40,41) coupled with the loss of hypothalamic neurons expressing prepro-Hcrt mRNA (5) or Hcrt peptides (6) in the postmortem human narcoleptic brain suggests that GHB is unlikely to act through a Hcrt-dependent mechanism to effect its therapeutic action. However, it should be remembered that some Hcrt cells remain in all postmortem human narcoleptic brains published to date (6). Perhaps the most direct test of the necessity of Hcrt neurons for GHB actions would be to assess the effects of GHB in the Hcrt/ataxin-3 mouse model in which the Hcrt neurons completely degenerate (42).

These results also raise the broader issue of the role of $GABA_B$ receptors in sleep. In humans, the $GABA_B$ agonist baclofen (25 mg) administered before sleep significantly prolonged total sleep time and reduced time spent awake after sleep onset (43). In aged rats, the $GABA_B$ antagonist CGP 35348 increased the duration of non-REM, REM, and total sleep duration compared to saline-injected controls (44). Blockade of $GABA_B$ receptors in the thalamus by microdialysis of CGP 35348 or another $GABA_B$ antagonist, 2-OH-saclofen, did not affect wakefulness or total sleep time in freely moving cats, but deep slow wave sleep and the mean power of slow waves (<10 Hz) decreased while light slow wave sleep was increased (45). Microinjection of either the $GABA_A$ agonist muscimol and $GABA_B$ agonist baclofen into the basal forebrain induced an increase in slow-wave sleep and an inhibition of wakefulness, but only muscimol caused a decrease

in desynchronized sleep parameters (46). Microinjection of baclofen into the pedunculopontine tegmentum (PPT) suppressed spontaneous REM sleep in a dose-dependent manner (47). $GABA_B$ receptors have been implicated in presynaptic control of GABA release onto the histaminergic cells of the tuberomammillary nucleus (48), which may provide a neural substrate for the effects observed in vivo. Together, these results point to an underappreciated role for $GABA_B$ receptors in sleep.

Acknowledgments

This work was supported by NIH R01AG02584, R01 MH61755 and R01HL59658, and Orphan Medical, Inc.

References

1. de Lecea L, Kilduff TS, Peyron C, Gao X.-B, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett II FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA, 1998; 95:322–327.
2. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.
3. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–376.
4. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–451.
5. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
6. Thannickal T, Moore R, Y, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
7. Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18:9996–10,015.
8. Kilduff TS, Peyron C. The hypocretin/orexin ligand-receptor system: Implications for sleep and sleep disorders. Trends Neurosci 2000; 23:359–365.
9. Hungs M, Mignot E. Hypocretin/orexin, sleep and narcolepsy. Bioessays 2001; 23:397–408.
10. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24:726–731.
11. Steininger TL, Kilduff TS. Anatomy of the hypocretin/orexin system. In: de Lecea L, Sutcliffe JG, eds. The Hypocretins/Orexins as Integrators of Physiological Signals. New York: Kluwer Academic/Plenum Publishers, in press.
12. Nitz D, Siegel JM. GABA release in posterior hypothalamus across sleep-wake cycle. Am J Physiol 1996; 271:R1707–R1712.
13. Li Y, Gao XB, Sakurai T, van den Pol AN. Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. Neuron 2002; 36:1169–1181.

14. Yamanaka A, Muraki Y, Tsujino N, Goto K, Sakurai T. Regulation of orexin neurons by the monoaminergic and cholinergic systems. Biochem Biophys Res Commun 2003; 303:120–129.
15. Xie XM, Morairty SR, Yamanaka A, Sakurai T, Kilduff TS. Electrophysiological effects of GABAB agonists on hypocretin/EGFP neurons in mouse hypothalamic slices. Sleep 2004; 27:A14.
16. Moragues N, Ciofi P, Lafon P, Tramu G, Garret M. GABAA receptor epsilon subunit expression in identified peptidergic neurons of the rat hypothalamus. Brain Res 2003; 967:285–289.
17. Harthoorn LF, Van Eden CG, Buijs RM. Functional characteristics of melanin-concentrating hormone and hypocretin/orexin containing neurons revealed by gene expression profiling, 33rd Soc Neurosci Meeting, New Orleans, LA, 2003.
18. Van Cauter E, Plat L, Scharf MB, Leproult R, Cespedes S, L'Hermite-Baleriaux M, Copinschi G. Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young Men. J Clin Invest 1997; 100:745–753.
19. Scharf MB. Pharmacology of classic and novel hypnotic drugs: From molecular pharmacology to therapeutics. In: Langer SZ, Mendlewicz J, Racagni C, eds. Target Receptors for Anxiolytics and Hypnotics. Basel: Karger, 1992.
20. Anonymous. Xyrem approved for muscle problems in narcolepsy. FDA Consum 2002; 36:7.
21. Wong CG, Gibson KM, Snead OC, 3rd. From the street to the brain: neurobiology of the recreational drug gamma-hydroxybutyric acid. Trends Pharmacol Sci 2004; 25:29–34.
22. Xie X, Smart TG. Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating GABAB receptors. Eur J Pharmacol 1992; 212:291–294.
23. Kaupmann K, Cryan JF, Wellendorph P, Mombereau C, Sansig G, Klebs K, Schmutz M, Froestl W, van der Putten H, Mosbacher J, Brauner-Osborne H, Waldmeier P, Bettler B. Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. Eur J Neurosci 2003; 18:2722–2730.
24. Queva C, Bremner-Danielsen M, Edlund A, Ekstrand AJ, Elg S, Erickson S, Johansson T, Lehmann A, Mattsson JP. Effects of GABA agonists on body temperature regulation in GABA(B(1))−/− mice. Br J Pharmacol 2003; 140:315–322.
25. Andriamampandry C, Taleb O, Viry S, Muller C, Humbert JP, Gobaille S, Aunis D, Maitre M. Cloning and characterization of a rat brain receptor that binds the endogenous neuromodulator gamma-hydroxybutyrate (GHB). Faseb J 2003; 17:1691–1693.
26. Snead OC, 3rd. Ontogeny of gamma-hydroxybutyric acid. II. Electroencephalographic effects. Brain Res 1984; 317:89–96.
27. Pompeiano M, Cirelli C, Tononi G. Effects of sleep deprivation on fos-like immunoreactivity in the rat brain. Arch Ital Biol 1992; 130:325–335.
28. Grassi-Zucconi G, Menegazzi M, De Prati AC, Bassetti A, Montagnese P, Mandile P, Cosi C, Bentivoglio M. c-fos mRNA is spontaneously induced in the rat brain during the activity period of the circadian cycle. Eur J Neurosci 1993; 5:1071–1078.
29. Shiromani PJ, Kilduff TS, Bloom FE, McCarley RW. Cholinergically induced REM sleep triggers Fos-like immunoreactivity in dorsolateral pontine regions associated with REM sleep. Brain Res 1992; 580:351–357.
30. Merchant-Nancy H, Vazquez J, Aguilar-Roblero R, Drucker-Colin R. c-fos proto-oncogene changes in relation to REM sleep duration. Brain Res 1992; 579:342–346.
31. Yamuy J, Mancillas JR, Morales FR, Chase MH. C-fos expression in the pons and medulla of the cat during carbachol-induced active sleep. J Neurosci 1993; 13:2703–2718.
32. Scammell T, Gerashchenko D, Urade Y, Onoe H, Saper C, Hayaishi O. Activation of ventrolateral preoptic neurons by the somnogen prostaglandin D2. Proc Natl Acad Sci U.S.A. 1998; 95:7754–7759.
33. Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, Saper CB. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000; 20:8620–8628.
34. Scammell TE, Gerashchenko DY, Mochizuki T, McCarthy MT, Estabrooke IV, Sears CA, Saper CB, Urade Y, Hayaishi O. An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. Neurosci 2001; 107:653–663.
35. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat Neurosci 2002; 5:979–984.
36. Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammell TE. Fos expression in orexin neurons varies with behavioral state. J Neurosci 2001; 21:1656–1662.

37. Ko EM, Estabrooke IV, McCarthy M, Scammell TE. Wake-related activity of tuberomammillary neurons in rats. Brain Res 2003; 992:220–226.
38. Baldo BA, Gual-Bonilla L, Sijapati K, Daniel RA, Landry CF, Kelley AE. Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. Eur J Neurosci 2004; 19:376–386.
39. Satoh S, Matsumura H, Fujioka A, Nakajima T, Kanbayashi T, Nishino S, Shigeyoshi Y, Yoneda H. FOS expression in orexin neurons following muscimol perfusion of preoptic area. Neuroreport 2004; 15:1127–1131.
40. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
41. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
42. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001; 30:345–354.
43. Finnimore AJ, Roebuck M, Sajkov D, McEvoy RD. The effects of the GABA agonist, baclofen, on sleep and breathing. Eur Respir J 1995; 8:230–234.
44. Puigcerver A, van Luijtelaar EL, Drinkenburg WH, Coenen AL. Effects of the GABAB antagonist CGP 35348 on sleep-wake states, behaviour, and spike-wave discharges in old rats. Brain Res Bull 1996; 40:157–162.
45. Juhasz G, Emri Z, Kekesi KA, Salfay O, Crunelli V. Blockade of thalamic GABAB receptors decreases EEG synchronization. Neurosci Lett 1994; 172:155–158.
46. Manfridi A, Brambilla D, Mancia M. Sleep is differently modulated by basal forebrain GABA(A) and GABA(B) receptors. Am J Physiol Regul Integr Comp Physiol 2001; 281:R170–R175.
47. Ulloor J, Mavanji V, Saha S, Siwek DF, Datta S. Spontaneous REM sleep is modulated by the activation of the pedunculopontine tegmental GABAB receptors in the freely moving rat. J Neurophysiol 2004; 91:1822–1831.
48. Stevens DR, Kuramasu A, Haas HL. GABAB-receptor-mediated control of GABAergic nhibition in rat histaminergic neurons in vitro. Eur J Neurosci 1999; 11:1148–1154.

35
The Activity Profile of Hypocretin Neurons in the Freely Moving Rat

BORIS Y. MILEYKOVSKIY and LYUDMILA I. KIYASHCHENKO
Department of Psychiatry and Biobehavioral Science, Brain Research Institute University of California, Los Angeles, California, U.S.A.

JEROME M. SIEGEL
Neurobiology Research, VA GLAHS Sepulveda, UCLA Department of Psychiatry, David Geffen School of Medicine, Los Angeles, California, U.S.A.

I. Introduction

Since discovery in 1998 of the hypothalamic neuropeptide hypocretin (Hcrt), also known as orexin, the study of Hcrt behavioral function has mainly been carried out by using of c-Fos immunostaining. It has been found increased c-Fos expression during locomotion, active wakefulness and decreased number of c-Fos positive Hcrt neurons during quite wakefulness and even more so during NREM sleep. Results have been controversial for rapid eye movement (REM) sleep. This method has a number of important limitations, in particular insufficient temporal resolution and dissociation of c-Fos expression and neuronal firing (1,2). A number of major questions that cannot be addressed using c-Fos also still need to be addressed. First, what is the baseline activity of Hcrt neurons in the brain during wakefulness? Second, why does such small portion of entire Hcrt population show c-Fos expression in response on variety of behavioral challenges? Third, why does the medial part of Hcrt cell field express c-Fos more readily than the lateral portion? Finally, what is the activity of these cells during REM sleep, whether tonic or phasic.

Only the extracellular recording of Hcrt cells during these behaviors in freely moving animals can answer these questions. Two recent attempts were made to describe discharge patterns of perifornical-lateral hypothalamic (PFH-LH) neurons across S-W cycle (3,4). Unfortunately, the neurotransmitter phenotype of the recorded cells was not determined in either of these studies. Combining juxtacellular cell labeling, micropipette and microwire recordings with spike waveform analysis, we have developed electrophysiological criteria for the identification of Hcrt neurons and showed some behavioral correlates of their activity in freely moving rats (5).

II. Electrophysiological Identification of Hcrt Neurons

We used micropipette unit recording in the PFH-LH region followed by juxtacellular Neurobiotin labeling and immunostaining of Hcrt to determine the specific spike waveform profile of Hcrt neurons in anesthetized rats. Hcrt neurons were also identified

antidromically during electrical stimulation of the Ventral Tegmental Area (VTA). This latter technique allowed the identification of the same subset of Hcrt neurons during subsequent experiments in freely moving rats. The VTA receives dense hypothalamic projections and about 20% of PFH-LH cells that project to the VTA contain Hcrt. Therefore, VTA stimulation would be likely to antidromicaly activate a large percentage of Hcrt neurons.

We found that antidromically and immunohistochemically identified Hcrt neurons have broad spikes with long-lasing Later Positive Deflection (LPD) that distinguished them from adjacent antidromically identified PFH-LH cells. The spike LPD of Hcrt neurons ranged from 0.82 ms to 1.2 ms with a mean of 0.93 ± 0.01 ms ($n = 26$) and was significantly broader than the spike LPD of nonHcrt neurons ($t = 12.3$, $p < 0.0001$). Labeling and appropriate immunostaining of PFH-LH cells with LPD in this range allowed the practical identification of Hcrt neurons that responded antidromically to electrical stimulation of the VTA. To apply the LPD criterion for unit recording with microwires, we recorded Hcrt neurons with composite electrodes that consisted of a glass micropipette to which tungsten microwire (12.5 μm) was attached and allowed simultaneous recording through both electrodes. We have determined that the spike LPD for Hcrt neurons exceeds 0.56 ms during microwire recordings.

In freely moving rats, we found 9 PFH-LH neurons with spikes that met our criteria for Hcrt cells in anesthetized animals. All had (1) a spike LPD between 0.56 ms and 0.77 ms (Fig. 1a), (2) responded antidromically to VTA stimulation (Fig. 1b,c), and (3) were located in the PFH-LH. Discharge patterns of these Hcrt neurons were analyzed during different behavioral states.

III. Hcrt Neuronal Activity During Sleep

The finding that most patients suffering from narcolepsy with cataplexy have lost most Hcrt neurons suggests that this hypothalamic neuropeptide plays a crucial role in maintaining wakefulness (6,7). Indeed, intracerebroventricular injections of Hcrt-1 produce a dose-dependent increase in the duration of wakefulness and substantially reduce REM sleep and slow wave (SW) sleep in rats (8). Investigation of the relation between Hcrt cell activity and sleep in rats showed that c-Fos expression in Hcrt neurons is circadianly modulated and negatively correlates with SW and REM sleep amounts. In contrast, the number of Hcrt+/c-Fos+ neurons significantly increases during sleep deprivation. After sleep recovery or REM sleep rebound Hcrt neurons show reduced c-Fos expression (9–11).

Our electrophysiological studies of the activity of identified Hcrt neurons across the sleep-wakefulness (S-W) cycle in freely moving rats showed that these neurons discharged with maximal frequency during active waking (AW), strongly decrease their firing rates during quiet wakefulness (QW) and practically cease activity in SW as well as in tonic REM sleep (Fig. 1d and Fig. 2a–d). During the phasic periods of REM sleep, Hcrt neuronal discharge became more frequent and sometimes correlated with spontaneous muscle twitches. A similar discharge pattern of identified Hcrt cells across S-W cycle was described by Lee et al. (12) who recorded and labeled Hcrt neurons in head restrained rats. The firing rate of Hcrt neurons increased during EEG desynchronization and negatively correlated with EEG spectral power in delta,

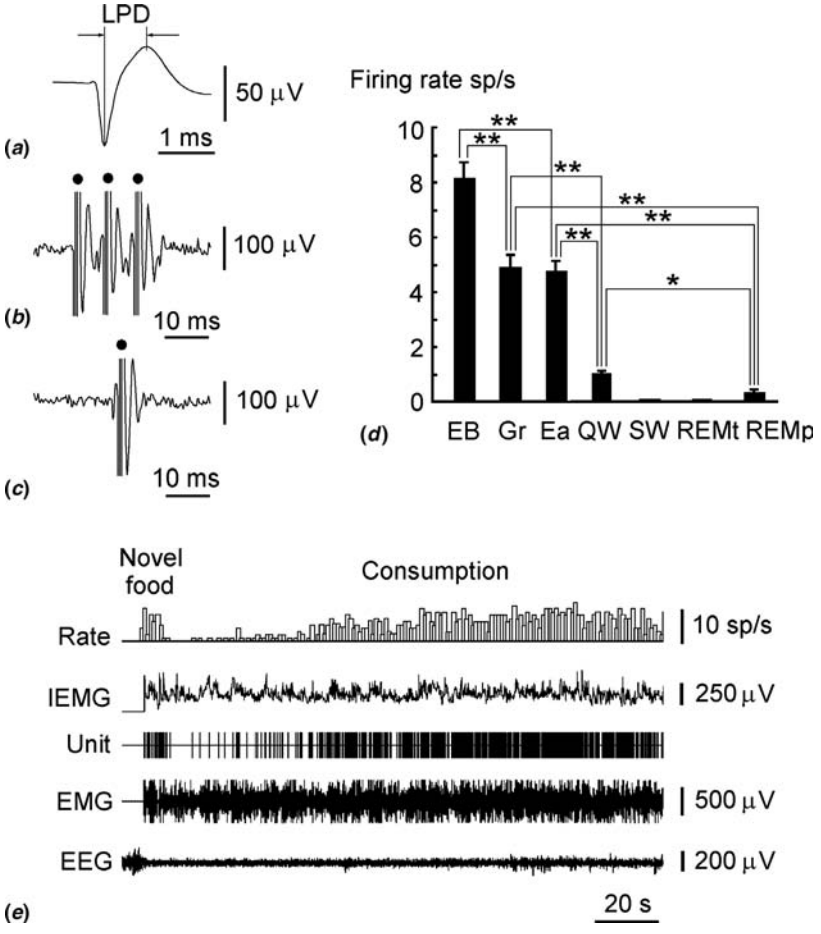


Figure 1 Example of electrophysiological identification of an Hcrt neuron in a freely moving rat and discharge pattern of Hcrt cells during different behavioral states. (***a***) An averaged spike waveform of the Hcrt neuron recorded with microwires. (***b***) Antidromic spikes of the Hcrt neuron to VTA train electrical stimulation. (***c***) Collision of orthodromic and antidromic spikes in axon of a Hcrt neuron. (***d***) Firing rate of Hcrt neurons in waking and sleep behaviors. (***e***) Transient decrease of Hcrt cell activity in response to the presentation of a novel food (chicken). During the observed decrease in firing rate, the rat sniffed, tasted and backed away from food. Hcrt cell firing accelerated in conjunction with the onset of food consumption. *Abbreviations*: AW, active waking; Ea, eating; EB, exploratory behavior; EEG, electroencephalogram; EMG, neck muscle electromyogram; Gr, grooming; QW, quiet waking; REMt and REMp, tonic and phasic REM sleep; Error bars indicate SEM; SW, slow wave sleep.

theta, alpha, and beta frequency bands (Fig. 2e–h). On the other hand, increased Hcrt cell activity was accompanied by an elevation of EEG spectral power in the gamma frequency band (Fig. 2i). It is worth noting that cortical desynchronization was always seen if Hcrt cells increased their firing rate to 1–2 Hz. This suggests that a low level

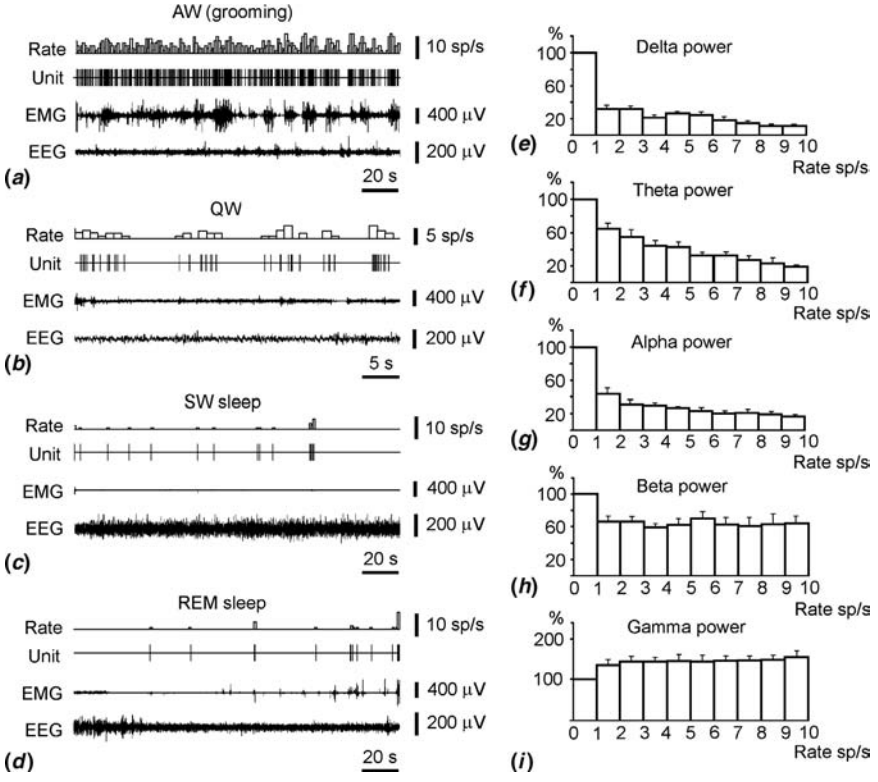


Figure 2 The discharge pattern of a representative Hcrt neuron across the sleep-wakefulness cycle in the freely moving rat and the alteration of EEG spectral power during periods of increased firing of Hcrt neurons. (***a***) High firing rates of an Hcrt neuron as seen during AW (grooming). (***b***) Reduced firing rate or cessation of Hcrt cell activity as seen in QW. (***c***) A further decrease or cessation of firing is seen during SW sleep. (***d***) Minimal firing rate is seen during the tonic phase of REM sleep. Brief Hcrt cell discharge bursts are correlated with muscle twitches during the phasic events of REM sleep. (***e–h***) The decrease of EEG powers in delta, theta, alpha, and beta frequency bands. (***i***) The increase of EEG power in gamma frequency band. Error bars indicate SEM. See abbreviations in Figure 1.

of the Hcrt neuronal activity is sufficient to induce and maintain ascending EEG activation. Hcrt neurons may contribute to cortical arousal through the excitation of ascending monoaminergic, cholinergic, reticular, and thalamocortical systems as well as through direct Hcrt projections to the cortex.

The behavioral correlates of Hcrt neuronal activity across the sleep cycle share similarities with those of the norepinephrine (13), serotonin (14), and histamine (15) cells to which Hcrt cells are reciprocally connected. However, unlike monoaminergic cells that predominantly show tonic "clock-like" activity during QW, Hcrt neurons have an irregular pattern of discharge sometimes alternating with periods of silence. During REM sleep, Hcrt neurons show sporadic discharges that sometimes correlate with muscle twitches, whereas most monoaminergic neurons cease firing completely.

The mechanisms underlying the suppression of Hcrt cell activity during sleep suggest that these neurons may be under GABAergic control from sleep-promoting regions of the preoptic area. Perfusion of the preoptic area with the GABA agonist muscimol or blockage of GABAergic function in the PFH-LH with bicuculline significantly increases the number of Hcrt+/c-Fos+ neurons (16,17). Thus, there is strong evidence for an active inhibition of Hcrt neurons during sleep.

IV. Hcrt Neuronal Activity and Feeding Behavior

Intracerebroventricular administration of Hcrt-1 and Hcrt-2 increases food consumption, suggesting an important role of these peptides in the regulation of feeding (18,19). c-Fos expression in Hcrt neurons is significantly increased when glucose level are decreased by the administration of insulin (20,21). Similarly, intracerebroventricular injections of ghrelin-releasing peptide that stimulates food intake as well as intraperitoneal administrations of intralipids producing robust elevation in the levels of triglycerides increase substantially the number of Hcrt+/c-Fos+ neurons (22,23). Both in rodent and primates, c-Fos expression in Hcrt neurons increases in responses to fasting and restricted feeding (24,25). Activation of Hcrt cells during these conditions suggests that they have a role in the maintenance of energy homeostasis through behavioral mechanisms, in particular triggering food-seeking behavior and regulating appetitive phase of feeding. This hypothesis is supported by the finding that the activity of Hcrt neurons markedly increases when food is anticipated under a condition of restricted feeding in wild-type mice. Additionally, Hcrt neuron-ablated mice display abnormal low food-anticipatory activity (26,27). Finally, c-Fos expression in Hcrt neurons after the administration of the GABA agonist muscimol into the nucleus accumbens shell (28) suggests that Hcrt systems may be also relevant to the regulation of the hedonic aspects of feeding.

In our electrophysiological studies, identified Hcrt neurons showed moderate activity when rat consumed a familiar food (Fig. 1d). Interestingly, however, they strongly decreased their firing rate during the period of the initial food aversion induced by the presentation of a novel food (Fig. 1e). This suppression of Hcrt cell discharges continued for 20–50 s despite strong EEG desynchronization and the presence of substantial motor activity that is usually associated with Hcrt cell activation. After tasting the novel (highly palatable) food, subsequent consumption was accompanied by a gradual elevation of Hcrt discharge frequency without any obvious EEG or motor alterations. Presented together, these results suggest that Hcrt neurons provide an important link between metabolic requirements and behaviors, modulating cortical arousal, motor activity, and regulating emotional/motivational aspects of feeding.

V. Hcrt Neuronal Activity and Motor Activity

The level of Hcrt-1 in the CSF of freely moving animals increases with motor activity (29,30). A greater number of Hcrt+/c-Fos+ neurons in cats is however observed during exploratory behavior when compared to stereotype locomotion on a treadmill (31). Similarly, we found that Hcrt cells discharge with a significantly higher frequency

during exploratory behavior when compared to grooming and eating (Fig. 1d). This is consistent with the idea that Hcrt neurons are involved in the integration of arousal and motor activity. Indeed, amphetamine and caffeine administration, which evoke a general behavioral activation, significantly increase the number of Hcrt+/c-Fos+ neurons in the medial part of the PFH-LH (32,33). We found that although the firing rate of Hcrt cells was correlated with the presence of motor activity (Fig. 3a,b), in some cases, this correlation was very weak or absent. Sound for example produced EEG desynchronization, elicited burst Hcrt cell discharge but did not substantially alter motor output (Fig. 3c). Similarly, behaviors with equal levels of motor activity could be accompanied by very different discharge frequencies of Hcrt neurons. As mentioned above, Hcrt cell activity decreased during food aversion despite the presence of substantially increased motor activity that manifested as repeated approaches and withdrawals. These results show that the discharge frequency of Hcrt neurons is modulated by sensory stimuli as well as emotional and/or motivational states independently of the activity of the motor system.

VI. Hcrt Neuronal Activity and Cataplexy

The data presented above suggest that Hcrt cell activation correlates with behaviors that may be associated with the triggering of cataplexy in human and animals. In particular, positive emotional events such as laughter, feelings of excitation or elation are the most frequently reported trigger for cataplexy in patients with narcolepsy (34,35). In contrast, sadness and pain very rarely induce cataplectic attacks. In narcoleptic dogs, cataplexy is triggered by the consumption of highly palatable food and by excited play, but not by regular food or a noxious stimuli (36,37). In rodents, triggering of cataplexy is most frequently linked to exploration, burrowing, seeking, and grooming (38,39), behaviors all associated with increased Hcrt cell activity (5). The excitation of the Hcrt system in the normal brain may thus appear to counter the activation of mechanisms that are responsible for a decrease in muscle tone and an inhibition of motor activity in response to positive emotional stimuli. The specific circuitry that underlie the emotional triggering of cataplexy is however unknown but may involve the interaction of mesocorticolimbic dopaminergic and Hcrt systems.

VII. Hcrt Neuronal Activity and Stress

Stress an unpleasant stimuli, has been suggested to activate Hcrt neurons in some conditions. Selected stress-like states (novelty-stress, immobilization, foot-shock) elicit c-Fos expression in Hcrt neurons (40–42). Analysis of the animal's behavior in these paradigms shows both a high level of arousal and of motor activity. Moreover, Hcrt neurons are activated by noxious stimuli and descending projections involved in descending regulation of analgesia (43). Thus, stress-inducing conditions related to motor activation and analgesia are accompanied by an excitation of the Hcrt system. In contrast, fear conditioning that is characterized by decreased motor activity and enhanced pain sensitivity does not elicit c-Fos expression in Hcrt neurons (41). Because c-Fos is not expressed in cells when the background activity is stable and

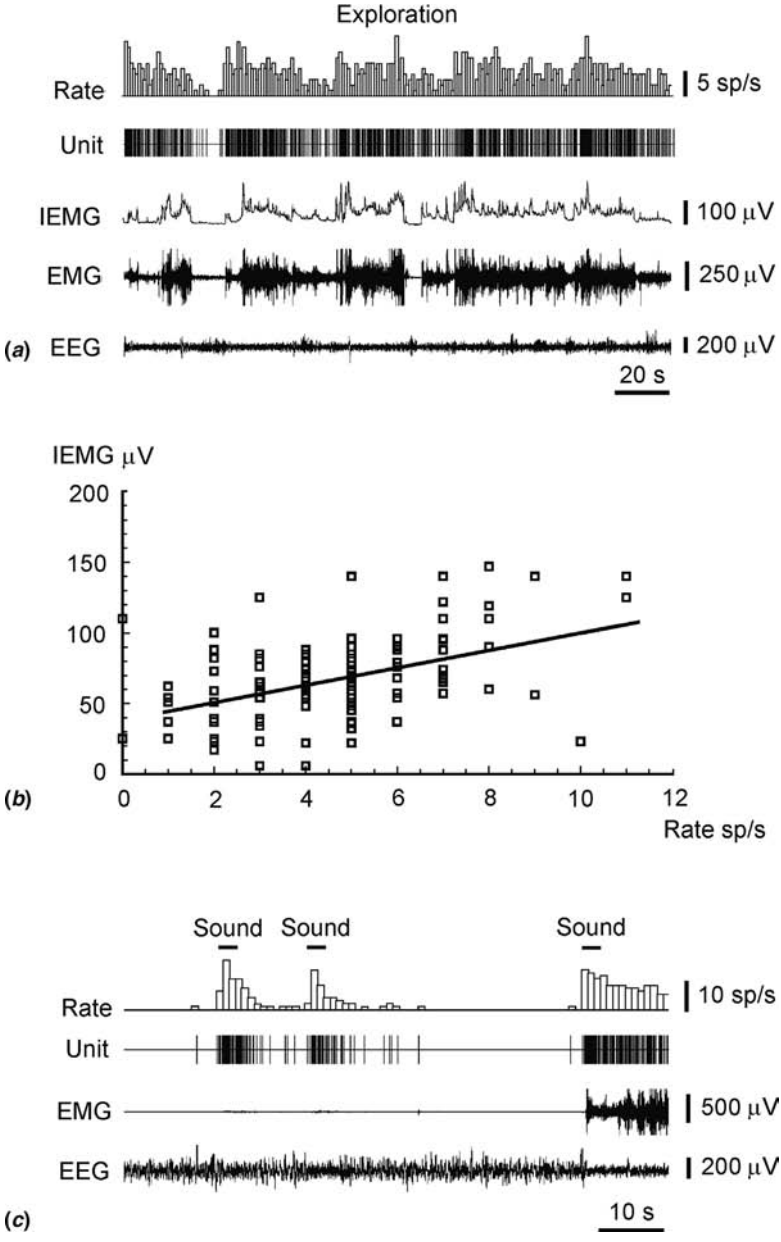


Figure 3 Example of the correlation between the firing rate of a Hcrt neuron and the amplitude of the neck EMG during exploratory behavior and short-lasting Hcrt cell excitation in response to sound stimuli. (***a***) The alteration of Hcrt cell firing rate, neck EMG, integrated neck EMG (IEMG), and EEG during exploratory behavior of the freely moving rat. (***b***) The correlation between Hcrt cell firing rate and the amplitude of the neck IEMG during exploratory behavior. (***c***) Sound stimuli induce Hcrt discharges independently of marked neck muscle activation. See abbreviations in Figure 1.

under inhibitory influences, it remains to be determined whether Hcrt cells keep their low baseline firing rate or if they are inhibited by fear. In this context, our data demonstrating strong reduction of Hcrt cell discharges during the presentation of novel food (neophobia) suggests an inhibition of Hcrt neurons by fear.

VIII. Hcrt Neuronal Activity and Psychotropic Drugs

Psychotropic drugs modulating dopaminergic tone have been shown to influence c-Fos activation in Hcrt cells. In rats, administration of methamphetamine and amphetamine increases the percent of Hcrt neurons expressing c-Fos in rats (9,32). The increased c-Fos expression correlates significantly with prolonged wakefulness and increased motor activity. Antipsychotic drugs clozapine, olanzapine, and risperidone also significantly increase the number of Hcrt+/c-Fos+ neurons after intraperitoneal administration. Interestingly, however, clozapine predominantly induces c-Fos in Hcrt neurons located in the lateral part of the PFH-LH, whereas other antipsychotics increase the number c-Fos positive Hcrt neurons in the medial hypothalamic region. Ziprasidone, haloperidol, and fluphenazine are ineffective in inducing c-Fos expression in Hcrt cells (32). Low doses of the wake-promoting drug modafinil significantly increase c-Fos immunoreactivity in Hcrt and tuberomammillary nucleus neurons suggesting that modafinil may promote wakefulness through both Hcrt and histamine systems (44). However, later studies showed that the wake-promoting effects of modafinil and methamphetamine are predominantly related to activation of dopaminergic system, because dopamine transporter knockout mice are unresponsive to administration of these drugs (45). Moreover, recent studies have shown that modafinil more effectively increases wakefulness in hypocretin knockout mice when compared to wild-type mice (46). Administration of mixed and selective dopamine receptor agonists increases the number of c-Fos positive Hcrt neurons. This effect must however involve transsynaptic mechanisms because Hcrt neurons rarely express dopamine receptors (47). Moreover, dopamine directly hyperpolarizes Hcrt cell membrane as shown in rodent slices (48).

Georgescu et al. (49) reported that 50% of Hcrt neurons express μ-opioid receptors and about 30% express c-Fos after chronic morphine administration or during acute withdrawal. These authors also found that Hcrt knockout mice develop attenuated morphine dependence. The number of Hcrt+/c-Fos+ neurons also increases in conditioned rats during exposure to environments previously paired with morphine, cocaine or food. Furthermore, there is a significant correlation between the amount of place preference and the number of Hcrt+/c-Fos+ neurons (50). These results suggest that Hcrt neurons may be involved in the action of some psychotropic drugs modulating arousal, emotional states and motor activity. The interaction of Hcrt neurons with structures of the limbic system, dopaminergic nuclei, and brainstem regions related to regulation muscle tone and motor output may underlie these effects.

IX. Conclusion

On the basic of juxtacellular labeling with Neurobiotin and immunohistochemical staining for Hcrt, we found that Hcrt neurons could be identified by antidromic

activation from the VTA and the presence of a broad LPD in their spike waveform. We found that Hcrt neurons discharge with maximal frequency during exploratory behavior and are relatively inactive in QW. During grooming and eating, Hcrt neurons have moderate and approximately equal levels of activity. Hcrt neurons are silent in SW sleep and tonic periods of REM sleep with occasional burst discharges that sometimes correlate with muscle twitches in phasic REM sleep. Responding to metabolic cues, Hcrt neurons may maintain energy homeostasis by triggering food-seeking behaviors. Some stress-like states accompanied by strong motor activity and analgesia increase the number of excited Hcrt neurons. Sensory stimuli and emotional cues may modulate Hcrt cell firing rate independently from cortical arousal and motor output. The presented data suggest that Hcrt neurons are involved in the regulation of emotional states, modulating arousal and motor activity, and are excited during conditions similar to those that trigger cataplexy in narcoleptic animals. Hcrt activity may thus be needed to counteract a state of physiological atonia trigerred by emotions.

References

1. Kovacs KJ. C-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int 1998; 33:287–297.
2. Hoffman GE, Lyo D. Anatomic markers of activity in neuroendocrine systems: are we all 'Fos-ed out'? J Neuroendocrin 2002; 14:259–268.
3. Alam MN, Gong H, Alam T, Jaganath R, McGinty D, Szymusiak, R. Sleep- waking discharge patterns of neurons recorded in the rat perifornical lateral hypothalamic area. J Physiol 2002; 538:619–631.
4. Koyama Y, Takahashi K, Kodama T, Kayama Y. State-dependent activity of neurons in the perifornical hypothalamic area during sleep and waking. Neuroscience 2003; 119:1209–1219.
5. Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 2005; 46:787–798.
6. Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
7. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
8. Piper DC, Upton N, Smith MI, Hunter AJ. The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. Eur J Neurosci 2000; 12:726–730.
9. Estabrooke IV, McCarthy MT, Ko E, Chou TC, et al. Fos expression in orexin neurons varies with behavioral state. J Neurosci 2001; 21:1656–1662.
10. Verret L, Goutagny R, Fort P, et al. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci 2003; 4:19.
11. Modirrousta M, Mainville L, Jones BE. Orexin and MCH neurons express c-Fos differently after sleep deprivation vs. recovery and bear different adrenergic receptors. Eur J Neurosci 2005; 21:2807–2816.
12. Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 2005; 25:6716–6720.
13. Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J Neurosci 1994; 14:4467–4480.
14. Jacobs BL, Fornal CA. Activity of brain serotonergic neurons in the behaving animal. Pharmacol Rev 1991; 43:563–578.
15. Vanni-Mercier G, Sakai K, Jouvet M. [Specific neurons for wakefulness in the posterior hypothalamus in the cat] Neurones specifiques de l'eveil dans l'hypothalamus posterieur du chat. C R Acad Sci III 1984; 298:195–200.
16. Satoh S, Matsumura H, Fujioka A, et al. Fos expression on orexin neurons following muscimol perfusion of preoptic area. Neuroreport 2004; 15:1127–1131.
17. Alam MN, Kumar S, Bashir T, et al. GABA-mediated control of hypocretin- but not melanin-concentrating hormone-immunoreactive neurons during sleep in rats. J Physiol 2005; 563:569–582.

18. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropertides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92:573–585.
19. Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatel MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J Endocrinol 1999; 160:R7-R12
20. Moriguch T, Sakurai T, Nambu T, Yanagisawa M, Goto K. Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. Neurosci Lett 1999; 264:101–104.
21. Cai XJ, Evans ML, Lister CA, et al. Hypoglycemia activates orexin neurons and selectively increases hypothalamic orexin-B levels: responses inhibited by feeding and possibly mediated by the nucleus of the solitary tract. Diabetes 2001; 50:105–112.
22. Lawrence CB, Snape AC, Baudoin FM, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 2002; 143:155–162.
23. Chang GQ, Karatayev O, Davydova Z, Leibowitz SF. Circulating triglycerides impact on orexin genis peptides and neuronal activity in hypothalamus. Endocrinology 2004; 145:3904–3912.
24. Kurose T, Ueta Y, Yamamoto Y, et al. Effects of restricted feeding on the activity of hypothalamic orexin (OX)-A containing neurons and OX2 receptor mRNA level in the paraventricular nucleus of rats. Regul Pept 2002; 104:145–151.
25. Diano S, Horvath B, Henryk F, Urbanski HF, Sotonyi P, Horwath TL. Fasting activates the nonhuman primate hypocretin(orexin) system and its postsynaptic targets. Endocrinology 2003; 144:3774–3778.
26. Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M. Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. J Neurosci 2004; 24:10493–501.
27. Akiyama M, Yuasa T, Hayasaka N, Horikawa K, Sakurai T, Shibata S. Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-oblated mice. Eur J Neurosci 2004; 20: 3054–3062.
28. Baldo BA, Gual-Bonilla L, Sijapati K, Daniel RA, Landry CF, Kelley AE. Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by $GABA_A$ receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. Eur J Neurosci 2004; 19: 376–386.
29. Kiyashchenko LI, Mileykovskiy BY, Maidment N, et al. Release of hypocretin (orexin) during waking and sleep states. J Neurosci 2002; 22:5282–5286.
30. Wu MF, John J, Maidment N, Lam HA, Siegel JM. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. Am J Physiol Regul Integr Comp Physiol 2002; 283:R1079–R1086.
31. Torterolo P, Sampogna S, Ramos O, Morales FR, Chase MH. Hypocretinergic system and motor activity in the cat. Sleep (Suppl.) 2004; 27:A5–A6.
32. Fadel J, Bubser M, Deutch AY. Differential activation of orexin neurons by antipsychotic drugs associated with weight gain. J Neurosci 2002; 22:6742–6746.
33. Murphy JA, Deurveilner S, Semba K. Stimulant doses of caffeine induce c-Fos activation in orexin/hypocretin-containing neurons in rat. Neuroscience 2003; 121:269–275.
34. Guilleminault C, Cataplexy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy. New York: Spectrum, 1976:125–143.
35. Krahn LE, Lymp JF, Moore WR, Slocumb N, Silber MH. Characterizing the emotions that trigger cataplexy. J Neuropsychiatry Clin Neurosci 2005; 17:45–50.
36. Mitler MM, Soave O, Dement WC. Narcolepsy in seven dogs. J Am Vet Med Ass 1976; 168:1036–1038.
37. Siegel JM, Nienhuis R, Fahringer H, et al. Neuronal activity in narcolepsy: identification of cataplexy related cells in the medial medulla. Science 1991; 262:1315–1318.
38. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–451.
39. Hara J, Beuckmann CT, Nambu T, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001; 30:345–354.
40. Espana RA, Valentino RJ, Berridge CW. Fos immunoreactivity in hypocretin-synthesizing and hypocretin-1 receptor-expressing neurons: effects of diurnal and nocturnal spontaneous waking, stress and hypocretin-1 administration. Neuroscience 2003; 121:201–217.

41. Zhu L, Onaka T, Sakurai T, Yada T. Activation of orexin neurones after noxious but not conditioned fear stimuli in rats. Neuroreport 2002; 13:1351–1353.
42. Winsky-Sommerer R, Yamanaka A, Diano S, et al. Interaction between the corticotrophin-releasing factor system and hypocretin (orexin): a novel circuit mediating stress response. J Neurosci 2004; 24:11439–11448.
43. Watanabe S, Kuwaki T, Yanagisawa M, Fukuda Y, Shimoyama M. Persistent pain and stress activate pain-inhibotory orexin pathways. Neuroreport 2005; 16:5–8.
44. Scammell TE, Estabrooke IV, McCarthy MT, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000; 20:8620–6628.
45. Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. J Neurosci 2001; 21:1787–1794.
46. Willie JT, et al. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience 2005; 130:983–995.
47. Bubser M, Fadel JR, Jackson LL, Meador-Woodruff JH, Jing D, Deutch AY. Dopaminergic regulation of orexin neurons. Eur J Neurosci 2005; 21:2993–3001.
48. Li Y, van den Pol AN. Direct and indirect inhibition by catecholamines of hypocretin/orexin neurons. J Neurosci 2005;25:173–183.
49. Georgescu D, Zachariou V, Barrot M, et al. Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. J Neurosci 2003; 23:3106–3111.
50. Harris G, Wimmer M, Aston-Jones G. Activity of orexin/hypocretin neurons corresponds with reward value of food- or morphine-paired stimuli. Program No. 576.14. Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2004.

36
Input and Output of Orexin/Hypocretin Neurons: Link Between Arousal Pathways and Feeding Behavior

TAKESHI SAKURAI
Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan and ERATO Yanagisawa Orphan Receptor Project, Japan Science and Technology Corporation, Tokyo, Japan

I. Introduction

The orexin (hypocretin) system has multiple upstream activators/inhibitors and downstream targets. This chapter discusses the input and output systems of orexin-producing neurons, and their physiological roles as a link between sleep/wakefulness states and energy homeostasis

II. Efferents of Orexin Neurons

A. Regulation of Behavioral States by Orexins

The finding of orexin (hypocretin) deficiency in narcoleptic patients suggests that orexin has an important role in the normal regulation of sleep/wakefulness (1,2). Orexin neurons might be especially important for stabilization of behavioral states, because the major symptom in narcolepsy is inability to maintain behavioral states, which results in sleep/wakefulness fragmentation. When orexin-A was injected intracerebroventricularly into rats during the light period, it caused increased wakefulness time and decreased REM and non-REM sleep time (3). This also caused prolonged wakefulness. In rats, Fos expression of orexin neurons is increased during the dark active period (4), and orexin level in cerebrospinal fluid also peaks during the dark active period and decreases during the light rest period (5). These observations suggest that orexin neurons are active during the active period and support wakefulness, and are inactive during the sleep period. The activities of monoaminergic neurons in the brain stem and hypothalmus are reportedly synchronized and strongly associated with behavioral states: they fire tonically during wakefulness, less during non-REM sleep, and not at all during REM sleep (6). This regulation might be at least in part by orexin neurons, which are also wake-active, because orexin neurons project to and excite histaminergic neurons in the tuberomammillary nucleus (TMN), noradrenergic neurons in the locus coeruleus (LC) and serotonergic neurons in the dorsal raphe (DR) (7–9), and the presence of OX_1R in the LC and OX_2R in the TMN and both receptors in the DR has been confirmed (10). Consistent with this hypothesis, isolated cells

from these nuclei are all activated by orexins in vitro (3,11,12). Among these systems, the effect of orexin on wakefulness is showed to be largely mediated by activation of the histaminergic system through activation of OX_2R in rodents. In rats, i.c.v. injection of orexin during the light period potently increases the wake period, and this effect is markedly attenuated by the H_1 antagonist, pyrilamine (12). Furthermore, the effect of orexin-A on wakefulness in mice is almost completely absent in H_1-receptor deficient mice (13). *OX_2R* knockout mice exhibit a narcoleptic phenotype, while *OX_1R* knockout mice show only mild fragmentation of behavioral states (14). Because OX_2R is abundantly expressed in the TMN, while OX_1R is heavily expressed in the LC, the TMN seems to be an important effecter site of orexin for sleep/wakefulness regulation. Orexin neurons also contain an additional neurotransmitter, dynorphin (15). Dynorphin in orexin neurons might inhibit GABAergic input to TMN neurons, and thus act in concert with orexin to increase the excitability of these neurons (16).

Orexin neurons also appear to act on the laterodorsal tegmental/pedunculopontine tegmental nucleus (LDT/PPT) cholinergic neurons, because orexin neurons project directly to the PPT/LDT nuclei (7) and direct injection of orexin-A into the LDT of cats results in an increase in wakefulness and a decrease in REM sleep (17). In addition, several reports showed that orexin induces long-lasting excitation of cholinergic neurons in the LDT (18). Recent work also shows that orexin inhibits cholinergic neurons in the PPT via activation of GABAergic local interneurons and GABAergic neurons in the substantia nigra pars reticulata (19). These results suggest that hypothalamic orexin neurons affect the activity of LDT/PPT cholinergic neurons directly and/or indirectly to appropriately regulate the activity of these cells to control behavioral states.

Orexins also have a strong direct excitatory effect on cholinergic neurons of the basal forebrain (20), which is also hypothesized to play an important role in behavioral and electrocortical arousal (21). These observations suggest that orexin neurons are active during the wakefulness period, and exert an excitatory influence on the basal forebrain cholinergic neurons and monoaminergic neurons in the brain stem to maintain arousal. Efferents of orexin neurons that regulate sleep/wakefulness states are summarized in Figure 1.

As discussed above, OX_2R is highly important for maintaining wakefulness. However, several findings indicate that signaling through OX_1R is also important for the regulation of sleep/wakefulness states. As mentioned before, *OX_2R* knockout mice exhibit characteristics of narcolepsy (22). Interestingly, *OX_1R* knockout mice do not have any overt behavioral abnormalities and exhibit only mild fragmentation of behavioral states (14). However, the behavioral and electroencephalographic phenotype of *OX_2R* knockout mice is less severe than that found in prepro-orexin knockout mice and double receptor knockout (*OX_1R*- and *OX_2R*-null) mice, which appear to have the same phenotype as prepro-orexin knockout mice. Willie et al. classified, using behavioral, electrophysiological, and pharmacological criteria, two distinct classes of behavioral arrest exhibited by mice deficient in orexin-mediated signaling (22). Both *OX_2R* and *prepro*-orexin knockout mice are similarly affected by behaviorally abnormal attacks of non-REM sleep ("sleep attacks") and show similar degrees of disrupted wakefulness. In contrast, *OX_2R* knockout mice are only mildly affected by cataplexy-like attacks of REM sleep, whereas orexin knockout mice are severely affected. Absence of *OX_2R* eliminates orexin-evoked excitation of histaminergic neurons in the

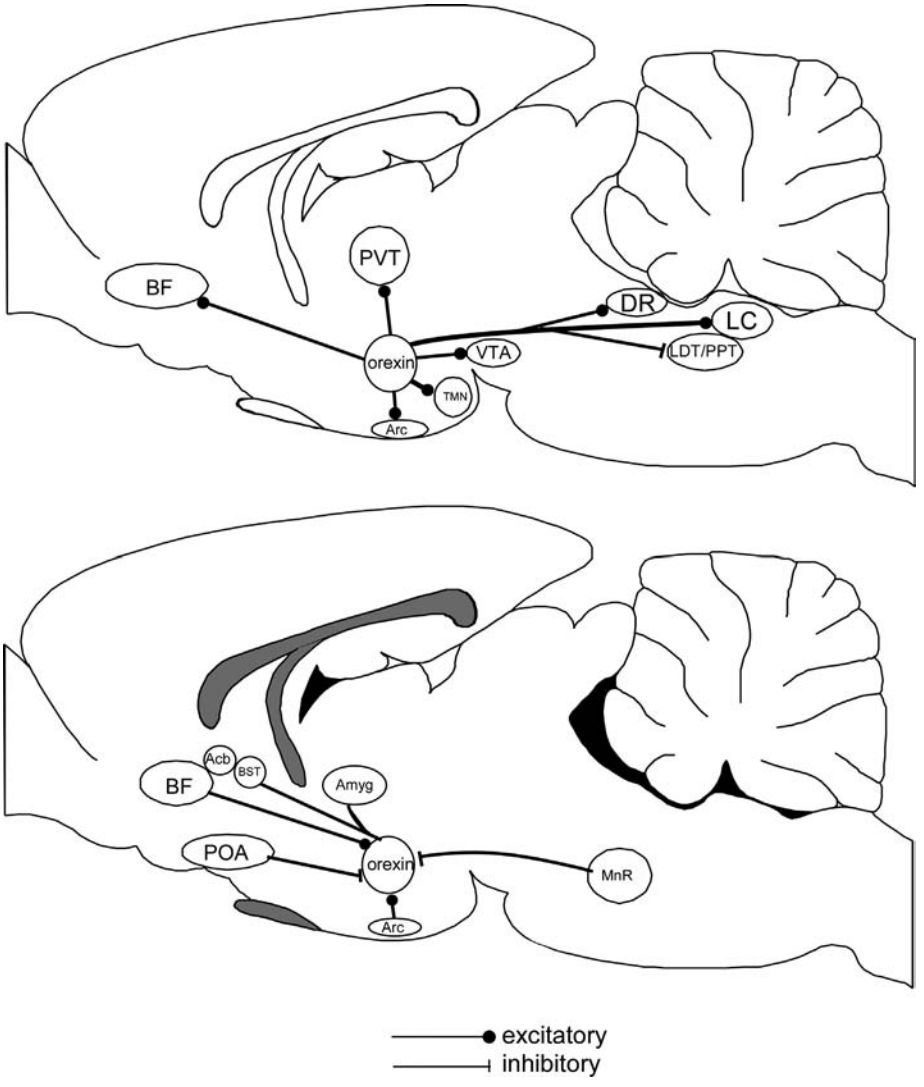


Figure 1 Schematic drawing showing major afferent (*top panel*) and efferent (*bottom panel*) systems of orexin neurons in the network implicated in the regulation of sleep/wakefulness states and feeding. Acb, nucleus accumbens; Amyg, amygdala; Arc, arcuate nucleus; BF, basal forebrain; BST, bed nucleus of the stria terminalis; PH, posterior hypothalamus; POA, preoptic area; TMN, tuberomammilary nucleus; LC, locus coeruleus; DR, dorsal raphe; MnR, median raphe; LDT, laterodorsal tegmental nucleus. VTA, ventral tegmenta area. Orexin neurons might be activated by the influences from the amygdala and the BST during wakefulness (43). Orexin neurons send excitatory innervation to monoaminergic cells in the brain stem, including LC, DR, VTA and TMN, and thereby elicit arousal (3,11,12). Orexin neurons also send excitatory projections to the Arc to regulate feeding (35). Serotonergic neurons in the MnR send inhibitory projections to orexin neurons; this innervation may act as a negative feedback system that limits activity of orexin neurons appropriately during wakefulness (43). During the sleep period, POA sleep-active cells send inhibitory projections to cholinergic cells and orexin neurons to silence these cells, which results in decreased activity of monoaminergic neurons in the brain stem.

hypothalamus, which gate non-REM sleep/wake transition. While normal regulation of wake/non-REM sleep transition depends critically upon OX_2R activation, the profound dysregulation of REM sleep control unique to the narcolepsy syndrome emerges from loss of signaling through both OX_2R-dependent and OX_1R-dependent pathways in mice. These observations suggest that OX_1R also has additional effects on sleep/wakefulness regulation. These findings suggest that despite the lack of an overt OX_1R phenotype, loss of signaling through both receptor pathways is necessary for a severe narcoleptic phenotype.

Thus, orexin neurons may be active during wakefulness, helping sustain activity in monoaminergic arousal regions (4), which in turn send inhibitory input to the ventrolateral preoptic area (VLPO) sleep-active neurons, and thereby further maintain wakefulness (23). In the absence of orexin, these arousal regions may have reduced activity during wakefulness states, resulting in an inappropriately low threshold for transition into non-REM sleep. Narcoleptic mice also have fragmented non-REM sleep; orexin-deficient mice have more frequent transitions between all states, as has been noted in human narcolepsy. Therefore, orexin neurons might also be necessary for maintenance of non-REM sleep.

B. Regulation of Feeding by Orexin Neurons

The pharmacological effect of orexin initially reported was stimulation of food intake (24). Although the efficacy of orexin was lower than that of neuropeptide Y (NPY), a well-known feeding peptide, it was as potent as other appetite-stimulating peptides such as melanin-concentrating hormone (MCH), and this effect of orexin was reproduced in several laboratories (25,26). On the other hand, administration of anti-orexin antibody or an OX_1R -selective antagonist reduced food intake (27,28), and *prepro-orexin* knockout mice and transgenic mice lacking orexin neurons ate less than control wild-type mice (14,29). Moreover, an OX_1R-selective antagonist reduced food intake and ameliorated obesity of leptin-deficient *ob/ob* mice (30). Then what is the role of orexin in the regulatory mechanism of feeding behavior and energy homeostasis?

An increase in adiposity results in a higher circulating leptin level, which crosses the blood-brain barrier to suppress feeding via neurons that express the signal-transducing leptin receptor, Ob-Rb (31,32) The arcuate nucleus (Arc), which contains neuropeptide Y (NPY)/agouti-related protein (AgRP)-coexpressing neurons and proopiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART)-coexpressing neurons, is a major site of leptin's action and is regarded as an important region that regulates feeding behavior (33). NPY/AgRP neurons stimulate feeding, while POMC/CART neurons suppress feeding. Leptin-mediated inhibition of NPY/AgRP neurons and excitation of POMC/CART neurons are thought to be the major mechanism of suppression of feeding by leptin (33,34). Orexin neurons densely project to the Arc (7,8,35) (Fig. 2), and Fos expression was induced in NPY neurons of the Arc by ICV injection of orexin, suggesting that orexin-stimulated feeding may occur at least partly through NPY pathways (35) (Fig. 1b). Indeed, the orexin-A-induced increase in food intake was partly inhibited by prior administration of BIBO3340, a NPY-Y1 receptor antagonist, in a dose-dependent manner (35). These experiments suggest that orexin-stimulated food intake is at least partially mediated

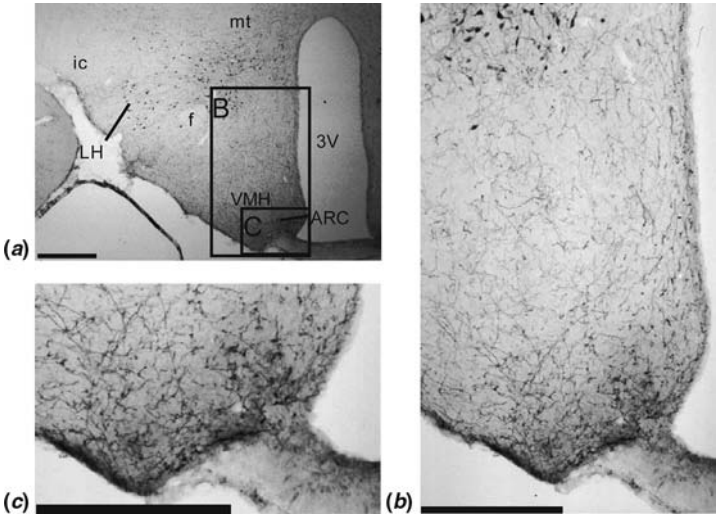


Figure 2 Orexin neurons in LHA innervate the arcuate nucleus. (***a***) Adult rat brain section stained by anti-orexin antiserum. Bregma-3.3 mm. LHA, lateral hypothalamic area; VMH, ventromedial nucleus of the hypothalamus; ARC, arcuate nucleus; 3V, third ventricle; mt, mammilothalamic tract; ic, internal capsule; f, fornix. Orexin-immunoreactive cell bodies are distributed within LHA. (***b, c***) High power view of region indicated in rectangle in panel (***a***). Note the abundant orexin-ir fibers in ARC and VMH. Bars equal 500 mm.

by activation of NPY neurons. However, because 60 μg of an NPY antagonist (which completely abolished NPY-induced feeding) only partially (50%) abolished orexin-induced feeding, other pathways by which orexin induces feeding might exist. This pathway might include inhibition of gluco-receptive, POMC-expressing neurons (36). Orexin-mediated arousal might be also important to support feeding behavior.

C. Importance of Co-Localized Factors in Orexin Neurons

Orexin neurons reportedly express several other neurotransmitters, including neuronal activity-regulated pentraxin (NARP) (37), glutamate (38), and dynorphins (15). Additional studies of *orexin/ataxin-3* mice, in which orexin neurons are genetically ablated, and *prepro-orexin* knockout mice under identical environmental and genetic conditions may clarify the physiological relevance of these factors (39). However, sleep state patterns of *orexin/ataxin-3* mice revealed by simultaneous EEG/EMG recording were very similar to that of *prepro-orexin* knockout mice, despite some differences in the genetic background (29). The similarity of these two mouse models further demonstrates the importance of orexins in the regulation of the sleep/wake state. Our observations also suggest that, although orexin-containing neurons produce other neuromodulators, orexin is the one that is important for the regulation of the sleep/wake state by these neurons.

On the other hand, these two mouse models have a difference in metabolic abnormality. *Orexin/ataxin-3* mice of under the C57BL/6J + DBA2 mixed genetic

background showed late-onset obesity, although these mice do not show obesity under the pure C57BL/6J background (39). In the case of *prepro-orexin* knockout mice, however, no significant difference in body weight between genotypes was observed under either the pure C57BL/6J background or the mixed (C57BL/6J + DBA2) background, although they showed a mild tendency toward obesity under both the mixed or pure C57/BL6 background. The difference of body weight gain between *prepro-orexin* knock out mice and *orexin/ataxin-3* mice under the same genetic background (C57BL/6J +DBA2) suggests that the more severe feeding and metabolic abnormalities of *orexin/ataxin-3* mice as compared with *prepro-orexin* knockout mice might be due to loss of other neurotransmitters in the orexin neurons that regulate metabolic activity.

III. Afferents of Orexin Neurons

A. Regulation of Orexin Neuronal Activity

Until recently, little was known about the factors that influence the activity of orexin neurons. Recent electrophysiological studies have identified several activators and inhibitors of orexin neurons. By recording from hypothalamic slices of transgenic mice expressing green fluorescent protein (GFP) only in orexin neurons, it was shown that agonists of ionotropic glutamate receptors (AMPA and NMDA) excite orexin neurons, whereas glutamate antagonists (AP-5, CNQX or NBQX) reduce their activity (40,41). These results indicate that orexin neurons are tonically activated by glutamate. Although orexin has little direct effect on the activity of orexin neurons, it increases this glutamate signaling by acting on presynaptic terminals (40). This mechanism may reinforce and coordinate the activity of orexin neurons in the LHA.

Several researchers have hypothesized that monoamines excite orexin neurons, forming positive feedback loops that would maintain wakefulness (42), but our electrophysiological studies showed just the opposite; both noradrenaline and serotonin hyperpolarize and inhibit GFP-expressing orexin neurons (40,41) (Fig. 3). Histamine has no effect on orexin neurons. These observations suggest that orexin neurons receive negative feedback from noradrenergic and serotonergic neurons. We also found that orexin neurons showed various responses to carbachol. Carbachol induced depolarization in 20% (20/104) of orexin neurons examined, and induced hyperpolarization in 3% (3/104) of orexin neurons examined during current clamp recording (43). The remaining population of orexin neurons did not detectably respond to carbachol. These findings suggest that functional heterogeneity exists among orexin neurons. The positive and negative regulation by cholinergic neurons might have roles in the regulation of orexin neurons according to vigilance states.

B. Neuronal Input to Orexin Neurons

We recently performed a retrograde tracing study using a genetically encoded tracer in mice (44). We generated transgenic mouse lines expressing a fusion protein consisting of human ICAM signal sequence, GFP and the non-toxic C-terminal fragment of tetanus toxin (TTC) (IGFP::TTC) exclusively in orexin neurons. This fusion protein was previously shown to be selectively transferred to interconnected neurons in a retrograde direction, and transported to the cell bodies of higher order neurons (45). This approach allowed us to visualize, at single-cell resolution, neurons that send synaptic

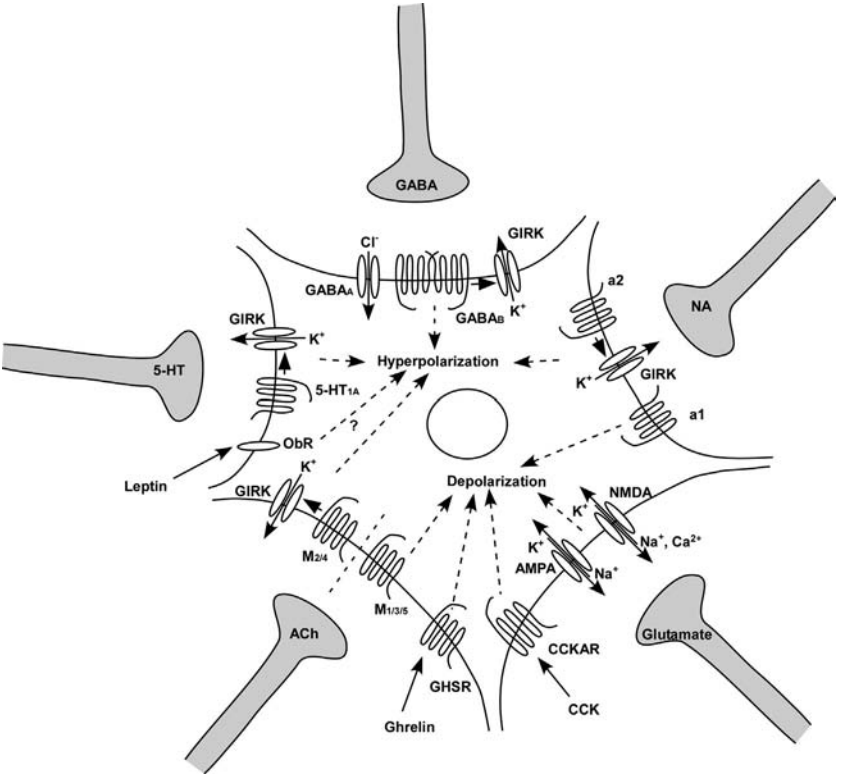


Figure 3 Schematic drawing of afferent system of orexin neurons estimated from electrophysiological experiments (41,51,57). NA, noradrenaline; ACh, acetylcholine; 5-HT, serotonin; GIRK, G-protein coupled inward rectifier potassium channel; $[Ca^{2+}]_i$, intracellular calcium concentration. NA, 5-HT, and leptin hyperpolarized orexin neurons, while ghrelin depolarized them. ACh showed various responses. In a group of orexin neurons, Ach evoked depolarization, while it evoked hyperpolarization in a minor population of orexin neurons.

projections to orexin neurons, by visualizing GFP in interconnected neural circuits. We identified GFP-positive cells in multiple specific brain regions, including basal forebrain cholinergic neurons, GABAergic neurons in the VLPO, and serotonergic neurons in the median raphe (MnR) and paramedian raphe (PMnR) nucleus. We also observed many neurons that are positive for GFP-ir in the dorsomedial hypothalamic nucleus (DMH) of the transgenic mice. Since the DMH receives direct and indirect SCN input, the SCN could send indirect input to orexin neurons via the DMH neurons to regulate the circadian timing of sleep, although we did not observe GFP-ir cells in the SCN, suggesting that direct SCN projections to orexin neurons are sparse.

We also found many labeled neurons in regions associated with emotion: we found many labeled neurons in the amygdalara, infralimbic cortex, shell region of the nucleus of accumbens, lateral septum and the bed nucleus of the stria terminalis (BST). Input from these regions might be important in regulating the activity of

orexin neurons upon emotional stimuli to evoke emotional arousal or fear-related responses. In fact, the importance of these inputs is readily apparent in the defense response, or "fight or flight" response: in an awake and freely moving condition, telemeter-indwelling orexin knockout mice showed diminished cardiovascular and behavioral responses to emotional stress in the resident-intruder paradigm (46). The neural input from the limbic system to orexin neurons might be important for pathophysiology of cataplexy, because generally positive emotional stimuli are known to trigger cataplexy in human narcolepsy patients. This implies that orexin neurons may play a role in the physiological responses associated with emotions in humans. Consistent with this, local injection of orexin into the PPT strongly inhibited REM-related atonia in the cat (19). Therefore, it is hypothesized that emotional stimuli increase orexin release in the PPT to prevent muscle atonia in wild type animals. Orexin neurons might inhibit cholinergic neurons in the PPT via GABAergic interneurons.

The innervations from the limbic system might also be important to maintain activity of orexin neurons during the active period by conveying various emotional stimuli to orexin neurons. Input to orexin neurons are summarized in Figure 4.

The input to orexin neurons from the limbic system might also be involved in the regulation of feeding behavior. The perception of food is processed in the limbic system. This information may be conveyed to orexin neurons to increase arousal and appetite. In fact, food perception often evokes cataplexy in narcoleptic dogs (food-elicited cataplexy) (47). Food-elicited cataplexy in narcoleptic dogs, in which the

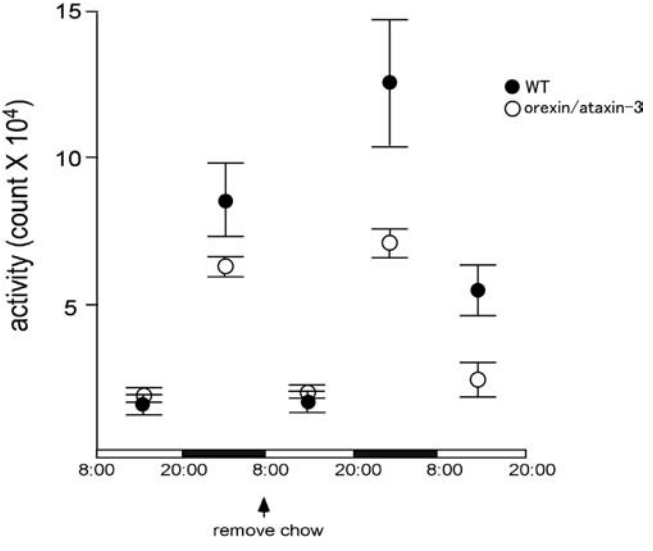


Figure 4 Impaired adaptive behavior of orexin neuron-ablated (*orexin/ataxin-3* transgenic) mice during food deprivation. Cumulative locomotor activities of male hemizygous *orexin/ataxin-3* mice (Tg, $n = 6$) and their weight-matched wildtype littermates (WT, $n = 6$) in home cages. After chows are removed, the wildtype animals respond with increased locomotor activity during both dark and light period. Such an adaptive response is impaired in the *orexin/ataxin-3* mice.

orexin signaling system is disrupted, suggests that orexin signaling is physiologically activated upon perception of food, and this system is necessary to evoke proper feeding behavior and appetite.

C. Regulation of Orexin Neurons by Humoral Factors Related to Energy Homeostasis

The altered energy homeostasis in human narcolepsy patients suggests roles of orexin in the regulation of energy homeostasis (48,49). The finding of decreased caloric intake (50) combined with an increased body mass index (48) suggests that narcolepsy patients have a feeding abnormality with reduced energy expenditure or a low metabolic rate, and orexin neurons have a role in the regulation of energy homeostasis. Consistently, orexin neuron-ablated mice show hypophagia and late-onset obesity (29).

Electrophysiological studies on orexin neurons showed that, in addition to monoamines and acetylcholine, peripheral humoral factors related to energy metabolism also influence the activity of orexin neurons; activity of isolated orexin neurons is inhibited by glucose and leptin, and stimulated by ghrelin (51). Consistently, orexin expression of normal and *ob/ob* mice is negatively correlated with changes in blood glucose, leptin, and food intake. These findings are consistent with the idea that orexins have a role in the regulation of feeding and energy homeostasis (24).

Then, what are the physiological roles of the regulation of orexin neurons by humoral factors? Proper maintenance of arousal during food searching and intake of an animal is essential for its survival. Therefore, the two vital physiological processes - feeding and sleep/wake behavior have to be appropriately coordinated. When faced with a negative energy balance due to reduced food availability, mammals respond behaviorally with phases of increased wakefulness and locomotor activity that support food seeking [52–56]. The discovery that orexin neurons are regulated by peripheral metabolic cues suggests that they might have important roles in the molecular and physiological basis of this evolutionarily conserved phenomenon. During starvation, orexin neurons might be activated by low leptin and glucose levels, along with ghrelin level. These mechanisms may directly modulate the activity of orexin neurons according to appetite and body energy stores. Indeed, we found that transgenic mice in which orexin neurons were ablated failed to respond to fasting with increased wakefulness and activity (51).

These findings indicate that orexin neurons provide a crucial link between energy balance and arousal. These properties might allow orexin neurons to promote alertness in a hungry animal.

IV. Summary

Orexins (hypocretins), which were initially identified as endogenous peptide ligands for orphan G-protein coupled receptors, have been shown to have an important role in the regulation of feeding behavior. Subsequently, the discovery of orexin deficiency in narcolepsy patients suggested that orexins are highly important factors for sleep/wakefulness regulation. Studies of efferent and afferent systems of orexin-producing neurons suggest interactions between these neurons and arousal centers in the

brainstem as well as important feeding centers in the hypothalamus. Electrophysiological studies have shown that orexin neurons are regulated by humoral factors, including leptin, glucose, and ghrelin as well as monoamines and acetylcholine. Orexin neurons have functional interactions with hypothalamic feeding pathways and monoaminergic/cholinergic centers to provide a link between peripheral energy balance and the CNS mechanisms that coordinate sleep/wakefulness and energy homeostasis.

V. Conclusion

Activity of orexin neurons appears to be strongly influenced by an animal's nutritional state. When faced with a negative energy balance due to reduced food availability, mammals respond behaviorally with phases of increased wakefulness and alertness that would presumably enhance the ability to find food in nature (55,56). Orexin neuron-ablated mice fail to exhibit fasting-induced arousal (51), suggesting that orexin neurons are necessary to evoke adaptive behavioral arousal during fasting. During periods of nutritional depletion, orexin increases arousal to reinforce food-seeking/feeding pathways. These mechanisms may be important in maintenance of prolonged wakefulness during the active period, and in the regulation of energy homeostasis that helps to ensure survival in nature, but may counteract attempts to treat obesity by food restriction.

Orexin neurons might be also activated by emotional stimuli from the limbic system. This system might be important to maintain long periods of wakefulness throughout the day.

Acknowledgments

This study was supported in part by a grant-in-aid for scientific research from The 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan; University of Tsukuba Project Research; the Uehara Memorial Foundation; and the ERATO from the Japan Science and Technology Agency Anorexia Nervosa Research Funds from the Japanese Ministry of Health, Labor and Welfare.

References

1. Peyron C, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 9:991–997.
2. Thannickal TC, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
3. Hagan JJ, et al. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. Proc Natl Acad Sci USA 1999; 96(19):10911–10916.
4. Estabrooke IV, et al. Fos expression in orexin neurons varies with behavioral state. J Neurosci 2001; 21:1656–1662.
5. Yoshida Y, et al. Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. Eur J Neurosci 2001; 14(7):1075–1081.
6. Vanni-Mercier G, Sakai K, Jouvet M. Neurons specifiques de l'eveil dans l'hypothalamus posterieur du chat. C R Acad Sci III 1984; 298:195–200.

7. Peyron C, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18(23):9996–10015.
8. Date Y, et al. Orexins orexigenic hypothalamic peptides interact with autonomic neuroendocrine and neuroregulatory systems. Proc Natl Acad Sci USA 1999; 96(2):748–753.
9. Nambu T, et al. Distribution of orexin neurons in the adult rat brain. Brain Res 1999; 827(1–2): 243–260.
10. Marcus JN, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol 2001; 435(1):6–25.
11. Nakamura T, et al. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. Brain Res 2000; 873(1):181–187.
12. Yamanaka A, et al. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochem Biophys Res Commun 2002; 290(4):1237–1245.
13. Huang ZL, et al. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA 2001; 98(17):9965–9970.
14. Willie JT, et al. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Ann Rev Neurosci 2001; 24:429–458.
15. Chou TC, et al. Orexin (hypocretin) neurons contain dynorphin. J Neurosci 2001; 21:RC168.
16. Eriksson KS, et al. Orexin (hypocretin)/dynorphin neurons control GABAergic inputs to tuberomammillary neurons. Eur J Neurosci 2004; 19:1278–1284.
17. Xi M, Morales FR, Chase MH. Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. Brain Res 2001; 901:259–264.
18. Takahashi K, et al. Effects of orexin on the laterodorsal tegmental neurones. Psychiatry Clin Neurosci 2002; 56(3):335–336.
19. Takakusaki K, et al. Role of orexinergic orijections to the midbrain involved in the control of locomotion and postural muscle tone. Abstr 34 th Meeting for Sci Neurosci 2004.
20. Eggermann E, et al. Orexins/hypocretins excite basal forebrain cholinergic neurones. Neuroscience 2001; 108(2):177–181.
21. Alam MN, et al. Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. J Physiol 1999; 521:679–690.
22. Willie JT, et al. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron 2003; 38(5): 715–730.
23. Gallopin T, et al. Identification of sleep-promoting neurons in vitro. Nature 2000; 404(6781):992–995.
24. Sakurai T, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998; 92(4):573–585.
25. Edwards CM, et al. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J Endocrinol 1999; 160:R7–R12.
26. Haynes AC, et al. Effects of single and chronic intracerebroventricular administration of the orexins on feeding in the rat. Peptides 1999; 20:1099–1105.
27. Yamada H, et al. Inhibition of food intake by central injection of anti-orexin antibody in fasted rats. Biochem Biophys Res Commun 2000; 267:527–531.
28. Haynes AC, et al. A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. Regul Pept 2000; 96(1–2):45–51.
29. Hara J, et al. Genetic ablation of orexin neurons in mice results in narcolepsy hypophagia and obesity. Neuron 2001; 30(2):345–354.
30. Haynes AC, et al. Anorectic thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. Regul Pept 2002; 104(1–3):153–159.
31. Rivest S. Does circulating leptin have the ability to cross the blood-brain barrier and target neurons directly? Endocrinology 2002; 143:3211–3213.
32. Friedman JM. Obesity in the new millennium. Nature 2000; 404:632–634.
33. Elias CF, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 1999; 23(4):775–786.
34. Hakansson ML, Meiste rB. Transcription factor STAT3 in leptin target neurons of the rat hypothalamus. Neuroendocrinology 1998; 68(6):420–427.
35. Yamanaka A, et al. Orexin-induced food intake involves neuropeptide Y pathway. Brain Res 2000; 24:404–409.

36. Muroya S, et al. Orexins (hypocretins) directly interact with neuropeptide Y, POMC and glucose-responsive neurons to regulate Ca^{2+} signaling in a reciprocal manner to leptin: orexigenic neuronal pathways in the mediobasal hypothalamus. Eur J Neurosci 2004; 19(6):1524–1534.
37. Reti IM, et al. Selective expression of Narp a secreted neuronal pentraxin in orexin neurons. J Neurochem 2002; 82(6):1561–1565.
38. Abrahamson EE, Leak RK, Moore RYT. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. Neuroreport 2001; 12:435–440.
39. Hara J, Yanagisawa M, Sakurai T. Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. Neuroscience Letters 2005. In Press.
40. Li Y, et al. Hypocretin/orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. Neuron 2002; 36(6):1169–1181.
41. Yamanaka A, et al. Regulation of orexin neurons by the monoaminergic and cholinergic systems. Biochem Biophys Res Commun 2003; 303(1):120–129.
42. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24:726–731.
43. Sakurai T, et al. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. Neuron 2005; 46(2):297–308.
44. Sakurai T, et al. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. Neuron 2005; 42(2):297–308.
45. Maskos U, et al. Retrograde trans-synaptic transfer of green fluorescent protein allows the genetic mapping of neuronal circuits in transgenic mice. Proc Natl Acad Sci USA 2002; 99(15):10120–10125.
46. Kayaba Y, et al. Attenuated defense response and low basal blood pressure in orexin knockout mice. Am J Physiol Regul Integr Comp Physiol 2003; 285:R581–593.
47. Reid MS, et al. Neuropharmacological characterization of basal forebrain cholinergic stimulated cataplexy in narcoleptic canines. Exp Neurol 1998; 151(1):89–104.
48. Schuld A, et al. Increased body-mass index in patients with narcolepsy. Lancet 2000; (355):1274–1275.
49. Honda Y, et al. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. Sleep 1986; 9:254–259.
50. Lammers GJ, et al. Spontaneous food choice in narcolepsy. Sleep 1996; 19:75–76.
51. Yamanaka A, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron 2003; 38(5):701–713.
52. Borbely AA. Sleep in the rat during food deprivation and subsequent restitution of food. Brain Res 1977; 124:457–471.
53. Danguir J, Nicolaidis S. Dependence of sleep on nutrients' availability. Physiol Behav 1979; 22: 735–740.
54. Dewasmes G, Duchamp C, Minaire Y. Sleep changes in fasting rats. Physiol Behav 1989; 46: 179–184.
55. Itoh T, et al. Effects of 24-hr fasting on methamphetamine- and apomorphine-induced locomotor activities and on monoamine metabolism in mouse corpus striatum and nucleus accumbens. Pharmacol Biochem Behav 1990; 35:391–396.
56. Challet E, Pevet P, Malan A. Effect of prolonged fasting and subsequent refeeding on free-running rhythms of temperature and locomotor activity in rats. Behav. Brain Res 1997; 84:275–284.
57. Muraki Y, et al. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. J Neurosci 2004; 24(32):7159–7166.

37
Human Leukocyte Antigen and Narcolepsy: Present Status and Relationship with Familial History and Hypocretin Deficiency

LING LIN and EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

As discussed in other chapters, narcolepsy is a common neurological disorders affecting 0.02% to 0.16% of the population (with cataplexy present). Although narcolepsy only occasionally occurs in multiplex families and monozygotic twin pairs are most commonly discordant, familial risk is increased. One to two percent of first-degree relatives are affected with narcolepsy-cataplexy, a 10 to 40-fold increase compared to general population prevalence (1).

One of the major genetic factors predisposing to narcolepsy is located within the HLA complex. Juji and Honda (2–4) were the first to recognize the existence of such an association (Table 1). The association was first recognized with the HLA class I allele Bw35 (2) in Japanese, a weak association that was later shown to be indirectly due to linkage disequilibrium with HLA-DR2 in this ethnic group. In Caucasian, the weak association secondary to linkage disequilibrium with HLA-Class I is with HLA-B7 (5). In 1984, the same group studied 40 narcolepsy patients and found them all to be HLA-DR2 and DQ1 positive (4). This tight DR2 and DQ1 association was almost immediately confirmed in Europeans and North American samples of mostly Caucasian origin (4–8).

The existence or not of genuine, HLA-DR2 negative, narcolepsy-cataplexy cases was hotly debated (10, see Table 1). In African Americans, a study found that up to 30% of patients were HLA-DR2 negative; all were DQ1 positive (11). Additional work in the area of HLA gene genotyping/sequencing found that in most narcolepsy cases the specific HLA-DR2, DQ1 haplotype Dw2 (as defined using Mixed Leukocyte culture), later characterized as DRB1*1501, DQA1*0102, DQB1*0602, was involved (12).

Further association and HLA region sequencing studies have now shown that a major reason for the lower association in African American was a difference in linkage disequilibria between DR2 and DQB1*0602 in this ethnic group (13–15). The primary HLA narcolepsy association is with HLA-DQB1*0602, a subtype of DQ1 not uncommonly associated with other DR subtypes in African Americans.

Table 1 An Historical Account of HLA Studies in Narcolepsy

Year	Historical account	Author	Reference
1981	Weak association with HLA-Bw35 in Japanese patients	Juji	2
1983	100% DR2, DQ1 association in Japanese patients with cataplexy	Honda, Juji	3,4
1984	Weak HLA-B7 association in Caucasian patients	Seignalet, Billiard	5
1984–1986	Tight HLA-DR2 association is confirmed in many countries	Langdon, Poirier, Mueller-Eckhardt, Billiard	6–9
1986	The existence of rare narcolepsy patients without HLA-DR2 but cataplexy is reported outside Japan, leading to a controversy	Guilelminault, Grumet	10
1986	Dw2 or DRB1*1501, DQA1*0102, DQB1*0602, a specific DR2 DQ1 haplotype, is found in all Japanese patients	Honda	12
1987	67% DR2; 100% DQ1 association in African American patients	Neely	11
1992	Most African American patients are DQB1*0602 positive but not always DR2 positive	Matsuki	13
1992	Most DR2 negative Caucasian patients with cataplexy are DQB1*0602 negative	Mignot	14
1994	DQB1*0602, DQA1*0102 are better markers than DR2 in Caucasian and African American	Mignot	15
1998	DQB1*0602 homozygocity increase relative risk	Pelin	45
1999	TNF-alpha may predispose in addition to HLA genetic effects	Hohjoh	46
2000	Narcolepsy is tightly associated with hypocretin deficiency; most patients with hypocretin deficiency are DQB1*0602 positive	Nishino	33
2000	First report of DQB1*0602 negative subject with hypocretin deficiency; in this case, a mutation in the hypocretin gene is likely causative	Peyron	34
2001	Complex HLA association in narcolepsy; DQB1*0301 is a second susceptibility allele while DQB1*0601and DQB1*0501 are protective alleles	Mignot	25
2002	Second reports of DQB1*0602 negative subject with hypocretin deficiency; in these cases, no mutation in hypocretin genes was found	Mignot, Dalal	17,18
2004	Other polymorphisms in the HLA complex region may predispose to narcolepsy in addition to HLA-DR and DQ	Mignot	26

The discovery of hypocretin deficiency as the cause of most cases of HLA-DQB1*0602 positive narcolepsy-cataplexy cases has added a new dimension to the field. The search for an autoimmune basis of narcolepsy has been reinitiated, as a target is now known and likely to be hypocretin-producing neurons. Exceptionally rare HLA-DQB1*0602 negative cases with hypocretin deficiency have also been reported. This includes a case initially reported by Guilleminault et al. (10) as DR2 negative who was largely the object of controversies (later shown to be DQB1*0602 negative). In other cases, however, narcolepsy-cataplexy may occur without HLA positivity and hypocretin deficiency (14, 16–18). In this chapter, we will review current knowledge regarding the complex relationships existing between clinical picture, HLA and hypocretin status. In this chapter, recent studies and information in random, monozygotic twins, trio, and multiplex families narcolepsy cases are reported.

II. HLA Gene: Structure and Function

The products of major histocompatibility complex (MHC) genes, also called human leukocyte antigen (HLA) genes, are antigen-presenting molecules designed for the presentation of antigen fragments to the T-cell receptor. This leads to the subsequent modulation of immune responses directed toward specific antigens.

The HLA complex is located on the short arm of human chromosome 6 (6p21.3) and is classically separated into the class I, class III, class II, and extended class I and II regions. The HLA class I gene family has 6 members: HLA-A, HLA-B, HLA-C (polymorphic loci); HLA-E, HLA-F, and HLA-G (oligomorphic loci). Its main loci, HLA-A, -B, and -C bind and present peptides derived from the intracellular degradation of cytoplasmic proteins for presentation to CD8-positive T cells. HLA class I molecules thus play an essential role in the defense against intracellular infections.

The HLA class II region contains DRB1, DQA1, DQB1, DPA1, and DPB1; these encode the alpha and beta chains of a large variety of cell-surface heterodimeric glycoproteins. HLA class II molecules usually present peptides derived from extracellular sources to CD4-positive (helper) T lymphocytes, triggering antigen recognition. Amino acids located at key positions along the alpha-helical portions of these HLA heterodimers dictate which peptide antigens can or cannot bind and thus are efficiently presented. A single amino acid substitution in the binding pocket region of Class II molecules (as found in various polymorphic subtypes) typically alters the shape of the HLA-peptide binding pocket sufficiently to dramatically change its peptide-binding repertoire.

The hallmark of the HLA system is its remarkable polymorphism. The extremely high variability of HLA Class II molecules is due to amino acid substitutions within hypervariable segments present in the external domain of alpha and beta chains. As of October 2003, 377 DRB1 and 57 DQB1 alleles have been reported, including 57 subtypes of DRB1*04 allele and 21 subtypes of DRB1*15 alleles. The specific amino acid sequence of each subtype is critical in mediating disease susceptibility as well as the ability to present processed antigens to T cells. Fortunately, however, most of the subtypes are very rare and DQB1 for example has only approximately 16 frequent allele across populations.

HLA polymorphisms have been shown to influence susceptibility to infection and cancer, yet the most consistent associations have been observed with autoimmune diseases most notably HLA–B27 and ankylosing spondylitis (19); DR4 and rheumatoid

arthritis (20); DQB1*0302 and type I diabetes mellitus (21); DRB1*1501 and multiple sclerosis (22). Surprisingly for a disease that has not yet been shown to be autoimmune in nature, narcolepsy has one of the tightest associations with HLA.

III. HLA-DQB1*0602 Susceptibility and Narcolepsy: A Complex Association

In narcolepsy-cataplexy, the major susceptibility allele is HLA-DQB1*0602. This allele is common in controls across multiple ethnic groups (carrier frequency: 12–38%, Table 2). It is highly enriched if not almost always present (85–100%) in patients with cataplexy (Table 2). The specific HLA subtype (and not a polymorphism in linkage with it) is believed to be involved. DQB1*0602 should only be considered a susceptibility factor, not a causative mutation. Indeed, narcolepsy patients share exactly the same nucleotide sequences as normal controls in the HLA-DR and DQ regions (15, 23). We have also studied and typed multiple microsatellite markers (CARI, II, III, and IV) in the area immediately flanking DQA1 and DQB1 in 912 individuals and found the DQA1*0102-DQB1*0602 haplotype in the region to be identical in controls and narcolepsy patients (16,23,24, unpublished data for CARIV). Overall, these studies strongly suggest that DQB1*0602 is the major susceptibility allele for narcolepsy. This allele is always found with DQA1*0102, and most probably it is the functional DQA1*0102/DQB1*0602 heterodimer that is responsible for disease susceptibility. In Japanese and Caucasians, the extended HLA-DRB1*1501-DQA1*0102-DQB1*0602-haplotype is typically involved. Very rarely, other extremely uncommon recombinant haplotypes, for example DRB1*0301-DQA1*0102-DQB1*0602, can be involved (16). In African-American, the association is with DQA1*0102-DQB1*0602 and different DR alleles such as DRB1*1501, DRB1*1503, DRB1*1101, DRB1*1201, and DRB1*0806 are commonly involved (16).

More detailed HLA association studies have shown that the effect of DQB1*0602 is quantitatively the most important effect in term of relative risk but that other modulatory influences can be detected through more careful analysis (Table 2). We recently studied 420 patients and 1,087 control subjects from three ethnic groups (Caucasian, Japanese, and African-American) and found a strong increase in relative risk for subjects homozygous for DQB1*0602 (25, Table 2). In addition, specific DQB1*0602 heterozygotes had different levels of susceptibility, consistent with a *trans* effect of the other allele. Most notably, DQB1*0602/DQB1*0301 had a higher level of predisposition than other combinations. In contrast, DQB1*0601 and DQB1*0501 were found to be rather protective (Note that DQB1*0601 is a mostly Asian subtype, Table 2).

A. Single-Family Transmission Disequilibrium Test (TDT) Study

The complexity of the HLA association pattern is reminiscent of findings in other autoimmune diseases such as Celiac disease, or type I diabetes mellitus where both susceptibility and protective haplotypes are involved and multiple DRB1 and DQB1 alleles may modulate disease susceptibility. To extend our observation of trans effects for the non-DQB1*0602 haplotype, a joined study of eight research groups was initiated under the auspices of the 13th international histocompatibility workshop (26). High resolution

Table 2 Differential Susceptibility Effects of Various DQB1*0602 Positive Genotype Combinations on Narcolepsy

	Caucasians				African Americans				Japanese			
DQB1 alleles	Narcolepsy n = 238(%)	Control n = 146(%)	OR	p	Narcolepsy n = 77(%)	Control n = 243(%)	OR	p	Narcolepsy n = 105(%)	Control n = 698(%)	OR	p
0602/0602	43 (18.1)	3 (2.1)	10.51	0.000	18 (23.4)	9 (3.7)	7.93	0.000	16 (15.2)	5 (0.7)	24.92	0.000
0602/0301	49 (20.6)	4 (2.7)	9.20	0.000	12 (15.6)	8 (3.3)	5.42	0.000	20 (19.0)	6 (0.9)	27.14	0.000
0602/0501	12 (5.0)	2 (1.4)	3.82	0.113	5(6.5)	3 (1.2)	5.56	0.031	2 (1.9)	5 (0.7)	2.69	0.510
0602/0601	0	0	n.a.	n.a.	0	0	n.a.	n.a.	8 (7.6)	15 (2.1)	3.76	0.005
0602/other DQB1	109 (45.8)	25 (17.1)	4.09	0.000	37 (48.1)	56 (23.0)	3.09	0.000	59 (56.0)	54 (7.7)	15.30	0.000
non 0602/non 0602	25 (10.5)	112 (76.7)	0.04	0.000	5 (6.5)	167 (68.7)	0.03	0.000	0	613 (87.8)	0.00	0.000

Note that the highest susceptibility risk occurs for subjects homozygous for DQB1*0602 whereas risk of developing narcolepsy in non DQB1*0602 subtype is almost zero. DQB1*0602/DQB1*0301 also have much higher risks than other combinations, most notably in Caucasians and Japanese. In contrast, combinations with DQB1*0501 or DQB1*0601 are rather protective. n.a. = not applicable.
Source: Modified from Ref. 25.

Alleles/Haplotypes	Trans	Non-trans	% trans	p-value
Non DRB1*1501, DQB1*0602 parents (129 families)*				
DQB1*0301	44	23	66	0.01
DRB1*0407	6	0	100	0.03
DQB1*0501	9	20	31	0.06
DRB1*0407-DQB1*0301	6	0	100	0.01
Non DRB1*0407, DQB1*0301	38	23	62	0.04
DRB1*0101-DQB1*0501	6	12	33	0.22
DRB1*1001-DQB1*0501	0	2	0	0.48
other DRB1-DQB1*0501	3	6	33	0.5
Non DQB1*0602, non DQB1*0301 parents (70 families)**				
DQB1*0501	6	16	27	0.04

* or **: Analysis of transmitted alleles from non DRB1*1501-DQB1*0602 and non DRB1*1501-DQB1*0602/DQB1*0301 parents respectively
T*: transmitted hyplotype

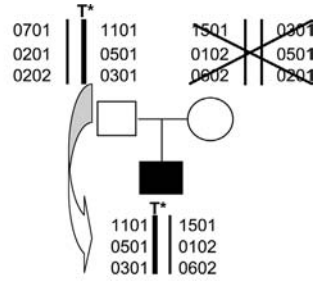


Figure 1 Single parent TDT analysis in DQB1*0602 heterozygous proband families.

typing of HLA-DRB1 and DQB1 alleles and Transmission Disequilibrium tests were performed in 198 trio families (proband with narcolepsy) from multiple ethnic groups including African-American, American and European Caucasian, Mexican Mestizos and Japanese Asian. As expected, a large primary transmission of DRB1*1501, DQB1*0602 alleles and haplotypes was observed (85–89%, $p < 0.00001$). Because narcolepsy is very strongly associated with DQB1*0602, all non-DQB1*0602 haplotypes carried by the DQB1*0602 positive parent have a large decreased risk of transmission independently of their genotype. To alleviate this effect, we only analyzed transmission of non- DQB1*0602 haplotypes from DQB1*0602 negative parents in the families with a DQB1*0602 positive proband (Fig. 1).

This novel analytic technique led to the interesting finding that the transmission of DQB1*0301 was indeed significantly increased as predicted from the heterozygote analysis (Table 2, Fig. 1). We also confirmed that the transmission of DQB1*0501 was significantly decreased in non-DQB1*0602 non-DQB1*0301 parents. A particularly strong effect of DRB1*0407-DQB1*0301 was also detected in trans of DQB1*0602.

We further analyzed transmission in 35 families without DQB1*0602 or DRB1*1501 in both parents. More surprisingly, we found that even in these non-DQB1*0602 cases DQB1*0301 was preferentially transmitted (26). This finding is in line with a previous observation indicating that similar alleles may predispose in trans of DQB1*0602 and in non-DQB1*0602 patients (25). This association suggests a similar HLA mediated susceptibility in non-DQB1*0602 narcolepsy. Importantly however, an earlier study by our group had not detected an increase in DQB1*0301 in non-DQB1*0602 positive subjects; rather an association with DQB1*0603/0607 was suggested (16). This last finding (DQB1*0301 susceptibility effect in non 0602 narcoleptic patient) may thus need to be further replicated.

B. HLA Association in Twin and Multiplex Family Cases

As mentioned in the introduction, narcolepsy is not a purely genetic disorder. This is suggested by the fact that most cases have a delayed adolescent onset and can be precipitated by sleep deprivation, stress, head trauma, and various medical conditions. The importance of nongenetic factors is also substantiated by monozygotic twin pair data (Table 3). Of 20 reported monozygotic twin pairs, only 7 (35%) are

Table 3 A Summary of Twin Pair Studied by Different Investigators

Study	Narcolepsy concordance	twin (n)	cataplexy	DR2/DQB1*0602	hypocretin-1
Imlah (1961)	concordant	1	+/+	n.d	n.d
Mitchel (1965)	discordant	1	+/−	n.d	n.d
Takahashi (1976)	discordant	1	+/−	n.d	n.d
Mamelak (1973)	concordant	1	+/+	n.d	n.d
Schrader (1980)	discordant	1	+/−	+	n.d
Asaka (1987)	discordant	2	+/−	n.d	n.d
Montplaisir (1987)	discordant	1	+/−	+	n.d
Douglas (1993)	concordant	1	+/+	−	n.d
Guilleminault (1993)	discordant	1	+/−	+	n.d
Pollmacher (1995)	discordant	2	+/−	+	n.d
Dahlitz (1994)	discordant	2	+/−	+	n.d
Dahlitz (1996)	concordant	1	+/+	+	n.d
Hayduk (1997)	concordant	1	+/+	−	n.d
Honda (2001)	concordant	1	+/+	+	n.d
Honda (2003)	discordant	1	+/−	+	n.d
Khatami (2004)	concordant	1	+/+	+	546/530
Dauvilliers (2004)	discordant	1	+/−	+	<40/530

n.d: not done; +/+: both have cataplexy; +/−: cataplexy only in one of the twins.
Source: From Refs. 1, 27–30.

concordant (1,27–30), suggesting the importance of environmental factors. All 7 available discordant pairs are HLA-DQB1*0602 positive (Table 3).

Surprisingly however, only 3 of 5 concordant twin pairs tested (60%) are HLA-DQB1*0602 positive (Table 3). This suggests that some non HLA-DQB1*0602 cases may have a particularly high genetic predisposition. In support of this hypothesis, studies in multiplex families have also reported lower DQB1*0602 positivity in selected multiplex families (1,18,31,32). We analyzed data from our own database and report findings in 31 Caucasian multiplex families (two members with narcolepsy and definite cataplexy) in Table 4. These results are compared to HLA typing data gathered from sporadic Caucasian narcoleptic subjects, who do not have a family history.

As shown in Table 4, HLA DQB1*0602 positivity was indeed significantly lower in familial cases (70%) than in random narcolepsy cases (87%), especially in families with a large number of affected individuals ($\geq$2, 56%) (Table 4). HLA typing data in these non-DQB1*0602 families did not support the concept of linkage to other HLA subtypes (1). This strongly suggests that non-HLA genetic factors may be involved in a subset of non HLA-DQB1*0602 cases.

The pattern of extended HLA haplotype segregation was also examined in DQB1*0602 positive multiplex families. We found that in general, these families had a smaller number of affected members, most often only 2 cases (Table 4). Interestingly, in some cases, the extended HLA-DQB1*0602 haplotype was not linked with narcolepsy and may have come from different branches of the family (1). This suggests that in these cases, multiple DQB1*0602 haplotypes (if not all DQB1*0602 alleles in the general population) in the family were equally predisposing to narcolepsy. Also, note that as for sporadic cases, homozygocity for DQB1*0602 was quite common in

Table 4 HLA-DQB1*0602 Positivity in Sporadic and Familial Caucasian Cases

	All samples	DQB1*0602 positive only with HLA-DRB1-DQB1 full typing available		Relative risk (and 95% CI if significant)
	DQB1*0602 positive n (%)	Homozygotes n (%)	Heterozygotes n (%)	Heterozygotes versus homozygous (versus unrelated controls or healthy relatives)
Sporadic cases				
Narcolepsy with typical cataplexy	498/574 (87%)[e]	52/277 (19%)	225/277 (81%)	3.1 (1.6–6.1)
Narcolepsy with atypical cataplexy	52/117 (44%)[e]	5/23 (22%)	18/23 (78%)	3.8 (1.2–11.7)
Narcolepsy without cataplexy (but SOREMPs)	31/93 (33%)[d]	1/8 (13%)	7/8 (87%)	1.9
Unrelated controls	358/1416 (25%)	11/160(7%)	149/160 (93%)	
Multiplex cases				
Narcolepsy with typical cataplexy	51/74 (70%)[b,e]	5/40 (12%)	35/40 (88%)	3.5
Narcolepsy with atypical cataplexy	15/30 (50%)	0/10 ((0%)	10/10 (100%)	0.0
Narcolepsy without cataplexy (but with SOREMPs)	6/9 (67%)	0/2 (0%)	2/2 (100%)	0.0
Narcolepsy with typical cataplexy in families with ≤ 2 affected in families	36/47 (77%)[a,e]	4/29(14%)	25/29 (86%)	3.9
Narcolepsy with typical cataplexy families with >2 affected in families	15/27 (56%)[b,c]	1/11 (9%)	10/11 (86%)	2.5
Healthy relatives	78/164 (48%)	2/51 (4%)	49/51 (96%)	

Note: Sporadic cases: random cases without family history. Data reported for multiplex cases include multiple cases in each multiplex family. Results are identical when only one proband per family (n = 35 families) is included (data not shown). Note that all cases tested without HLA-DQB1*0602 have normal CSF hypocretin-1.

Typical cataplexy is defined as muscle weakness triggered at least sometimes by laughing or joking.

[a] $p = 0.05$ vs. sporadic cases.

[b] $p < 0.001$ vs. sporadic cases.

[c] $p = 0.05$.

[d] $p = 0.05$ vs. unrelated controls or healthy relatives when appropriate.

[e] $p = 0.05$ vs. narcolepsy in ≤ 2 affected per family.

multiplex families with less than three affected (Table 4). These results are generally consistent with the notion that in many cases, these families have narcolepsy cases of similar etiologies as random sporadic cases. As seen later, the result is also consistent with the observation of hypocretin deficiency in these HLA positive multiplex family cases.

IV. HLA Alleles in Typical, Atypical Narcolepsy, and Idiopathic Hypersomnia

As noted in Table 4 and also as reported by many other investigators, the HLA association is very high (85–95%) only in narcolepsy cases with cataplexy, especially typical narcolepsy (triggered by laughing and joking). In patients without cataplexy or with doubtful cataplexy (atypical narcolepsy), HLA-DQB1*0602 frequency is also increased (33–44%), but many patients are DQB1*0602 negative. HLA typing results in patients with idiopathic hypersomnia are generally unremarkable and close to control allele frequency (Table 5). As we mentioned, a large number of control individuals also have the DQB1*0602 allele without having narcolepsy (12–38%). These results suggest increased disease heterogeneity with non-narcoleptic hypersomnias most likely rarely involving the same pathophysiology than narcolepsy-cataplexy. The increased HLA positivity in narcolepsy without cataplexy may result from both etiological heterogeneity and/or the possibility that without DQB1*0602, hypocretin deficiency and the disease process may be less severe.

V. HLA Association and Hypocretin-1 Deficiency

The discovery that hypocretin receptor-2 mutations causes canine narcolepsy led us to measure hypocretin-1 in the cerebrospinal fluid (CSF) of human narcoleptic patients (33) and initiate neurophathological studies (34). CSF hypocretin-1 and HLA typing results collected to date in randomly recruited Stanford patients, included on the basis of visiting a sleep clinic and being diagnosed with narcolepsy are reported in Table 5. As noted previously, HLA positivity and hypocretin deficiency are highest in cases with cataplexy (80–90%). Most importantly, however, HLA and hypocretin status are highly correlated with each other across all diagnostic categories, with almost all hypocretin deficient subjects being HLA positive (Table 5).

Additionally, we found that only 20% of patients with atypical or without cataplexy are hypocretin-deficient. This result is fully consistent with an increased HLA-DQB1*0602 positivity from 25% in controls to 30% to 50% in these cases (Table 5). It is also of interest to note that approximately 10% of subjects with classic, typical narcolepsy have normal CSF hypocretin-1 (Table 5). Similarly, HLA-DQB1*0602 frequency is still generally increased in subjects with narcolepsy and intermediate/normal CSF hypocretin-1 (Tables 5 and 6). This may suggest that even in subjects with normal CSF hypocretin, an HLA mediated susceptibility could be involved in at least some cases. Together with the fact many HLA-DQB1*0602 negative subjects may have DQB1*0301 and often normal CSF hypocretin-1, we have proposed the hypothesis that in some cases, (especially those without DQB1*0602 and/or without cataplexy), a partial lesion of the hypocretin system could be involved (18). In favor of this hypothesis, Thannickal et al. has reported that in one case without cataplexy, the number of cells was less decreased than in cases with cataplexy (35). We also found in rat studies that lesions of up to 73% of hypocretin producing cells only decrease CSF hypocretin-1 50% below normal levels (36), levels we would consider in the intermediate range in our study. It is possible that many cases have "partial hits" where the process is initiated but stops, only

Table 5 HLA Positivity and Hypocretin Deficiency in a Multiethnic, Randomly Selected, Sample of Patients and Controls Studied at Stanford

	Narcolepsy with typical cataplexy		Narcolepsy with atypical cataplexy		Narcolepsy without cataplexy		Hypersomnia		Control	
	n	%	n	%	n	%	n	%	n	%
Caucasian	578/822	70%	118/173	68%	96/133	72%	42/62	68%	1416/1921	74%
DQB1*0602	730/822	89%	88/173	51%	51/133	38%	11/62	18%	469/1921	24%
Low HCRT-1	132/157	84%	8/45	18%	10/54	19%	0/40	0%	0/30	0%
DQB1*0602/HCRT-1 $\leq$ 110 pg/mL	132/132	100%	7/8	88%	10/10	100%	0/0	0%	0/0	0%
DQB1*0602/ HCRT-1 $>$ 110 pg/mL	7/25	28%	16/37	43%	13/44	30%	8/40	20%	7/30	23%

Only randomly selected patients are included. Patients recruited because of DQB1*0602 negativity, due to family history or secondary cases are not included.
This sample is representative of patients consulting for narcolepsy or hypersomnia. Typical cataplexy is muscle weakness triggered at least sometimes by laughing or joking. HCRT-1 = CSF hypocretin-1
Narcolepsy without cataplexy requires a MSLT $\leq$ 8 minutes and $\geq$ 2SOREMPs. Hypersomnia may be with or without prolonged nocturnal sleep. See ICSD 2004.

Table 6 Hypocretin-1 Level: Relationship with HLA-DQB1*0602 in Narcolepsy and Control Subjects Across Ethnic Groups

		Hypocretin-1 (HCRT-1) and DQB1*0602 status					
		Primary narcolepsy cases with and without cataplexy (n = 362)				Unrelated control (n = 30)	
		Low HCRT-1 (n = 229)		Normal/intermediate HCRT-1(n = 132)		Normal HCRT-1 (n = 30)	
Author	Reference	DQB1*0602 +	DQB1*0602 −	DQB1*0602 +	DQB1*0602 −	DQB1*0602 +	DQB1*0602 −
Dalal et al., 2001 and 2002	17,47	10[a]	1				
Kanbayashi et al., 2002; Kubota et al., 2003; Tsukamoto et al., 2002	48–50	13		3	4		
Krahn et al., 2002	51	12		4	10		
Dauvilliers et al., 2003 and 2004	41,52	28		2	1		
Lecendreux et al., 2003	40	1					
Ebrahim et al., 2003[c]	53	14					
Khatami et al., 2004[d]	29			2			
Nishino et al., 2000; Hong et al., 2002; Mignot et al., 2002; Bassetti et al., 2003; Zuberi et al., 2004; Stanford group to date[b]	18,33,42, 54,55	154	3#	39	67	7	23
Total		231	4	50	82	7	23
% of corresponding sample		98%	2%	38%	62%	23%	77%

Patients with both typical and atypical cataplexy are included; Note that the samples included are not representative of a random population of narcoleptic patients; rather, they include more DQB1*0602 negative and familial cases than a randomly selected narcolepsy population sample explaining the slightly lower HLA positivity/hypocretin deficiency value; secondary narcolepsy cases are not included.

[a]DR2 tested; A single HLA negative subject was also reported later in a separate publication and was included in both studies.

[b]Includes mostly patients from Stanford University but also patients from Dr. Hong (Korea), Drs. Bassetti (Switzerland), Lammers (Netherland), and Nevsimalova (Chezk Republic)

[c]Only narcolepsy-cataplexy patients were included due to unusual definition of narcolepsy without cataplexy (only one SOREMP).

[d]Twin reports.

[e]Also includes a subject with early onset narcolepsy-cataplexy associated with a probable causal hypocretin gene mutation; see text for description of these expectionally rare subjects. Low CSF hypocretin-1: ≤110 pg/ml; Intermediate: 110 < levels ≤ 200 pg/ml; >200 pg/ml as described in Ref. 18.

leading to symptom when selected functionally important hypocretin projections are affected or lesions are complete.

Our results are fully consistent with data gathered by other investigators (Table 6). In this more heterogenous groups of subjects studied across ethnicities and cultural boundaries which include many atypical cases, an amazing 98% of all hypocretin deficient patients reported were HLA-DQB1*0602 positive. One of the four DQB1*0602 negative patients is an atypical case with a probable causative prepro-hypocretin mutation and disease onset at six months (34). The three other patients had a more regular peripubertal/adolescent onset, although in one case cataplexy triggers were atypical (severe cataplexy often unlinked to emotions, produced by a large variety of triggers including disgust and negative emotions) and in another case cataplexy was mild an infrequent. HLA typing in these three cases was unremarkable, except for the fact two cases had rare DRB1*04 haplotypes (DRB1*0407-DQB1*0301 and DRB1*0409-DQB1*0302), one being DQB1*0301 homozygotes and one was DQB1*0603 positive. Additional data on these exceptional cases will need to be collected to extend on this observation.

VI. Recent Autoimmune Studies

As discussed in other chapters and above, most HLA associated diseases are autoimmune in nature. The tight HLA association and hypocretin deficiency suggest that the immune system is involved in the destruction of hypocretin-producing cells. Disappointingly however, most attempts to date to demonstrate an autoimmune process have failed (37,38, Silber et al. in this volume). Chabas et al. searched for autoantibodies directed against hypocretin-1, hypocretin-2, and prepro-hypocretin in narcolepsy patients without obtaining significant results (37). Similarly, Taheri et al. used serum and CSF samples of 40 HLA-DQB1*0602 positive hypocretin deficiency patients and 120 healthy controls to search for antibodies reacting with dog, rat, and mice hypothalami. Samples studied included recent onset cases and patients with atypical narcolepsy, some without cataplexy. Occasional binding to the hypothalamus (and other regions), as evaluated by Western blots or immunocytochemistry was detected in some cases but no differences were noted with controls (38).

An exception to available negative data is the recent result by Smith et al. (39). In this recent study, immunoglobulin (Igs) purified from the sera of nine HLA positive patients with narcolepsy (two without cataplexy) and nine controls, were injected into BALB/c mice. Mice were killed 48 hours later and bladder detrusor muscle strips (2/mice) isolated. The resulting muscles were pharmacologically denervated and sensitivity to cholinergic M3 stimulation examined by application of carbachol. Increased sensitivity to carbachol (and endogenously released acetylcholine after electric field stimulation) was found in all narcolepsy cases. This effect was indirect, as direct application of purified Igs on the bladder did not produce the same effect, indicating whole-organism effects that in turn induced a secondary cholinergic hypersensitivity. The authors also noted behavioral changes in mice injected with narcolepsy but not control Igs but no changes in brain hypocretin content. Similar studies were also performed at Stanford using different mice strains, longer-term administration and in conjunction with pertusis toxin to open the blood brain barrier, with no obvious

behavioral and neurochemical changes (unpublished observation). The Smith et al. study, if replicated, suggests the existence of narcolepsy specific antibodies that may only be detectable using high sensitivity bioassays (39).

VII. Perspectives

Although results to date are rather negative in this area, the autoimmune hypothesis is still likely but may be difficult to demonstrate with current technical limitations, as discussed in Smith et al. (39). For example, target autoantigens on hypocretin cells may be of low abundance, the process may be short lasting. Alternatively, the immune system may be more directly involved in regulating susceptibility to an infectious agent damaging hypocretin cells. These general hypotheses are consistent with the complex genetic transmission and HLA association, the role of environmental triggers and the perpubertal onset. It is also consistent with the preliminary observations suggesting that intravenous immunoglobulin may reduce symptoms when applied close to disease onset, at a time when the autoimmune process would still be active (40–42).

Interestingly, the HLA-DQB1*0602 allele has strong susceptibility effect for narcolepsy but provides dominant protection against type I diabetes. To elucidate the molecular features underlying these contrasting genetic properties, Siebold et al. determined the crystal structure of the DQB1*0602 molecule (43). They found that the subtype could bind with high affinity—and thus likely present efficiently to the rest of the immune system—the most N-terminal portion of the preprohypocretin molecule [aminoacids 1–13]. Further crystallographic experiments suggested that the HLA class II binding pocket 4 may be fundamentally important to the difference between DQB1*0602 associated susceptibility and DQB1*06011 associated protection in term of polarity. Importanatly, however, we and Dr. Silber (this volume) could not find evidence for specific autoantibodies directed against preprohypocretin peptides,

Pocket	P4		P6			P9			
DQ Residues	13β	26β	9β	30 β	66α/69α*	37β	38 β	57β	Note
0102-'0602	G	L	F	Y	A	Y	A	D	major susceptibility allele, almost required
0301-0301	A	Y	Y	Y	L	Y	A	D	susceptibility effect in heterozygotes and possibly in non DQB1*0602 patients
0501-'0301	A	Y	Y	Y	L	Y	A	D	susceptibility effect in heterozygotes and possibly in non DQB1*0602 patients
0601-'0301	A	Y	Y	Y	T	Y	A	D	susceptibility effect in heterozygotes and possibly in non DQB1*0602 patients
0103-'0603/0607	G	L	Y	H	A	Y	A	D	possibly increased in non DQB1*0602 patients
0201-0202	G	L	Y	S	L	I	V	A	neutral
0501-0202	G	L	Y	S	L	I	V	A	neutral
0102-'0604	G	L	Y	H	A	Y	A	V	neutral
0103-'0601	A	Y	L	Y	A	D	V	D	protective in the presents of DQB1*0602
0101-'0501	G	G	Y	H	A	Y	V	V	protective in the presents of DQB1*0602

Note that all narcolepsy susceptibility subtypes share the same amino acid residues in pocket 9.
α denotes DQα chain; β denotes DQβ chain.
*: location number reported as aligned with DR1β and as reported by IMGM/HLA DQB1 sequence database

Figure 2 Amino acid residues of selected DQ heterodimers in peptide binding pocket 4, 6, and 9.

making it less likely it is involved as an antigen in the autoimmune process. We also noted that YAD residues in pocket 9 were the most discriminating residues across narcolepsy susceptibility and protective alleles (44, Fig. 2). We therefore rather favor the hypothesis that other unknown presented peptides with a primary interaction with DQB1*0602 and other DQB1 molecules are involved, with possible combined effects of multiple pocket sites and multiple alleles.

References

1. Mignot E. Genetic and familial aspects of narcolepsy. Neurology 1998; 50:S16–S22.
2. Juji T, Maeda H, Honda Y, Asaka A, Akizuki M, Kashiwagi H. An analysis of human B lymphocyte antigen system other than HLA-DR locus, and HLA typing for narcolepsy and SEL. In: Watanabe I, ed. Annual Report of Research Committee on Host Factors of Intractable Diseases, for the Year 1980. Tokyo:Ministry of Health and Welfare, 1981:141–147.
3. Honda Y, Asaka A, Tanaka Y, Juji T. Discrimination of narcolepsy by using genetic markers and HLA. Sleep Res 1983; 12:254.
4. Juji T, Satake M, Honda Y, Doi Y. HLA antigens in Japanese patients with narcolepsy. All the patients were DR2 positive. Tissue Antigens 1984; 24:316–319.
5. Seignalet J, Billiard M. Possible association between HLA-B7 and narcolepsy. Tissue Antigens 1984; 23:188–189.
6. Langdon N, Welsh KI, van Dam M, Vaughan RW, Parkes D. Genetic markers in narcolepsy. Lancet 1984; 2:1178–1180.
7. Poirier G, Montplaisir J, Decary F, Momege D, Lebrun A. HLA antigens in narcolepsy and idiopathic central nervous system hypersomnolence. Sleep 1986; 9:153–158.
8. Mueller-Eckhardt G, Meier-Ewert K, Schendel DJ, Reinecker FB, Multhoff G, Mueller-Eckhardt C. HLA and narcolepsy in a German population. Tissue Antigens 1986; 28:163–169.
9. Billiard M, Seignalet J, Besset A, Cadilhac J. HLA-DR2 and narcolepsy. Sleep 1986; 9(1 Pt 2):149–52.
10. Guilleminault C, Grumet C. HLA-DR2 and narcolepsy: not all narcoleptic-cataplectic patients are DR2. Hum Immunol 1986; 17:1–2.
11. Neely S, Rosenberg R, Spire JP, Antel J, Arnason BG. HLA antigens in narcolepsy. Neurology 1987; 37:1858–1860.
12. Honda Y, Juji T, Matsuki K, Naohara T, Satake M, Inoko H, Someya T, Harada S, Doi Y. HLA-DR2 and Dw2 in narcolepsy and in other disorders of excessive somnolence without cataplexy. Sleep 1986; 9:133–142.
13. Matsuki K, Grumet FC, Lin X, Gelb M, Guilleminault C, Dement WC, Mignot E. DQ (rather than DR) gene marks susceptibility to narcolepsy. Lancet 1992; 339:1052.
14. Mignot E, Lin X, Kalil J, George C, Singh S, Billiard M, Montplaisir J, Arrigoni J, Guilleminault C, Dement WC, et al. DQB1-0602 (DQw1) is not present in most nonDR2 Caucasian narcoleptics. Sleep 1992; 15:415–422.
15. Mignot E, Lin X, Arrigoni J, Macaubas C, Olive F, Hallmayer J, Underhill P, Guilleminault C, Dement WC, Grumet FC. DQB1*0602 and DQA1*0102 (DQ1) are better markers than DR2 for narcolepsy in Caucasian and black Americans. Sleep 1994; 17:S60–S67.
16. Mignot E, Kimura A, Lattermann A, Lin X, Yasunaga S, Mueller-Eckhardt G, Rattazzi C, Lin L, Guilleminault C, Grumet FC, Mayer G, Dement WC, Underhill P. Extensive HLA class II studies in 58 non-DRB1*15 (DR2) narcoleptic patients with cataplexy. Tissue Antigens. 1997; 49:329–341.
17. Dalal MA, Schuld A, Pollmacher T. Undetectable CSF level of orexin A (hypocretin-1) in a HLA-DR2 negative patient with narcolepsy-cataplexy. J Sleep Res 2002; 11:273.
18. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
19. Bowness P, Zaccai N, Bird L, Jones EY. HLA-B27 and disease pathogenesis: new structural and functional insights. Expert Rev Mol Med 1999; 26:1–10.
20. Buckner JH, Nepom GT. Genetics of rheumatoid arthritis: is there a scientific explanation for the human leukocyte antigen association? Curr Opin Rheumatol 2002; 14:254–259.

21. Pociot F, McDermott MF. Genetics of type 1 diabetes mellitus. Genes Immun 2002; 3:235–249.
22. Oksenberg JR, Barcellos LF, Cree BA, Baranzini SE, Bugawan TL, Khan O, Lincoln RR, Swerdlin A, Mignot E, Lin L, Goodin D, Erlich HA, Schmidt S, Thomson G, Reich DE, Pericak-Vance MA, Haines JL, Hauser SL. Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. Am J Hum Genet 2004; 74:160–167.
23. Ellis MC, Hetisimer AH, Ruddy DA, Hansen SL, Kronmal GS, McClelland E, Quintana L, Drayna DT, Aldrich MS, Mignot E. HLA class II haplotype and sequence analysis support a role for DQ in narcolepsy. Immunogenetics 1997; 46:410–417.
24. Lin L, Jin L, Lin X, Voros A, Underhill P, Mignot E. Microsatellite single nucleotide polymorphisms in the HLA-DQ region. Tissue Antigens 1998; 52:9–18.
25. Mignot E, Lin L, Rogers W, Honda Y, Qiu X, Lin X, Okun M, Hohjoh H, Miki T, Hsu S, Leffell M, Grumet F, Fernandez-Vina M, Honda M, Risch N. Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am J Hum Genet 2001; 68:686–699.
26. Mignot E, Lin L, Li H, Thomson G, Lathrop M, Thorsby E, Tokunaga K, Honda Y, Dauvilliers Y, Tafti M, Billiard M, Mayer G, Montplaisir J, Rouleau G, Temple H, Thorpy M, Martins B, Silva AM, Vazquez M, Alaez C, Gorodezky C, Schoch G, Lo B, Grumet FC. The 13th International Histocompatibility Working Group for Narcolepsy Joint Report. 13th IHWS Disease Component Joint Report. HLA 2004 Immunobiology of the Human MHC Proceedings of the 13th IHWC. ed: Hansen J. Dupont B. in press.
27. Honda M, Honda Y, Uchida S, Miyazaki S, Tokunaga K. Monozygotic twins incompletely concordant for narcolepsy. Biol Psychiatry 2001; 49:943–947.
28. Honda Y. Amonozygotic twin pair completely discordant for narcolepsy, with sleep deprivation as a possible precipitating factor. Sleep and Biological Rhythms 2003; 1:147–149.
29. Khatami R, Maret S, Werth E, Retey J, Schmid D, Maly F, Tafti M, Bassetti CL. Monozygotic twins concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 363:1199–1200.
30. Dauvilliers Y, Maret S, Bassetti C, Carlander B, Billiard M, Touchon J, Tafti M. A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology 2004; 62:2137–2138.
31. Guilleminault C, Mignot E, Grumet C. Family patterns of narcolepsy. Lancet 1989; 11:1376–1380.
32. Nevsimalova S, Mignot E, Sonka K, Arrigoni JL. Familial aspects of narcolepsy-cataplexy in the Czech Republic. Sleep 1997; 20:1021–1026.
33. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
34. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
35. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
36. Gerashchenko D, Murillo-Rodriguez E, Lin L, Xu M, Hallett L, Nishino S, Mignot E, Shiromani PJ. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Exp Neurol 2003; 184:1010–1016.
37. Chabas D, Taheri S, Renier C, Mignot E. The genetics of narcolepsy. Annu Rev Genomics Hum Genet 2003; 4:459–483.
38. Taheri S, Krempetz M, Jackson M, Paterno J, Mignot E. Investigation of the autoimmune basis of narcolepsy using western blot analysis of lateral hypothalamus protein extract with serum and cerebrospinal fluid (CSF) (abstr). Sleep 2003; 26:A285.
39. Smith AJ, Jackson MW, Neufing P, McEvoy RD, Gordon TP. A functional autoantibody in narcolepsy. Lancet 2004;364(9451):2122–4.
40. Lecendreux M, Maret S, Bassetti C, Mouren MC, Tafti M. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old boy. J Sleep Res 2003; 12:347–348.
41. Dauvilliers Y, Carlander B, Touchon J, Tafti M. Successful management of cataplexy with intravenous immunoglobulins at narcolepsy onset. Annals of Neurology 2004; 56(6):905–908.
42. Zuberi SM, Mignot E, Ling L, et al. Variable response to intravenous immunoglobulin therapy in childhood narcolepsy. Journal of Sleep Research 2004; 13(Suppl 1):828.

43. Siebold C, Hansen BE, Wyer JR, Harlos K, Esnouf RE, Svejgaard A, Bell JI, Strominger JL, Jones EY, Fugger L. Crystal structure of HLA-DQ0602 that protects against type 1 diabetes and confers strong susceptibility to narcolepsy. Proc Natl Acad Sci USA 2004; 101:1999–2004.
44. Lin L, Hungs M, Mignot E. Narcolepsy and the HLA region. J Neuroimmunol 2001; 117:9–20.
45. Pelin Z, Guilleminault C, Risch N, Grumet FC, Mignot E. HLA-DQB1*0602 homozygosity increases relative risk for narcolepsy but not disease severity in two ethnic groups. US Modafinil in Narcolepsy Multicenter Study Group. Tissue Antigens 1998; 51:96–100.
46. Hohjoh H, Nakayama T, Ohashi J, Miyagawa T, Tanaka H, Akaza T, Honda Y, Juji T, Tokunaga K. Significant association of a single nucleotide polymorphism in the tumor necrosis factor-alpha (TNF-alpha) gene promoter with human narcolepsy. Tissue Antigens 1999; 54:138–145.
47. Dalal MA, Schuld A, Haack M, Uhr M, Geisler P, Eisensehr I, Noachtar S, Pollmacher T. Normal plasma levels of orexin A (hypocretin-1) in narcoleptic patients. Neurology 2001; 56:1749–1751.
48. Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, Fujii Y, Nishino S, Shimizu T. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002; 11:273.
49. Kubota H, Kanbayashi T, Tanabe Y, Ito M, Takanashi J, Kohno Y, Shimizu T. Decreased cerebrospinal fluid hypocretin-1 levels near the onset of narcolepsy in 2 prepubertal children. Sleep 2003; 26: 555–557.
50. Tsukamoto H, Ishikawa T, Fujii Y, Fukumizu M, Sugai K, Kanbayashi T. Undetectable levels of CSF hypocretin-1 (orexin-A) in two prepubertal boys with narcolepsy. Neuropediatrics 2002; 33:51–52.
51. Krahn LE, Pankratz VS, Oliver L, Boeve BF, Silber MH. Hypocretin (orexin) levels in cerebrospinal fluid of patients with narcolepsy: relationship to cataplexy and HLA DQB1*0602 status. Sleep 2002; 25:733–736.
52. Dauvilliers Y, Baumann CR, Carlander B, Bischof M, Blatter T, Lecendreux M, Maly F, Besset A, Touchon J, Billiard M, Tafti M, Bassetti CL. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry 2003; 74:1667–1673.
53. Ebrahim IO, Sharief MK, de Lacy S, Semra YK, Howard RS, Kopelman MD, Williams AJ. Hypocretin (orexin) deficiency in narcolepsy and primary hypersomnia. J Neurol Neurosurg Psychiatry 2003; 74:127–130.
54. Hong SC, Leen-Kim, Park SA, Han JH, Lee SP, Lin L, Okun M, Nishino S, Mignot E. HLA and hypocretin studies in Korean patients with narcolepsy. Sleep 2002; 25:440–444.
55. Bassetti C, Gugger M, Bischof M, Mathis J, Sturzenegger C, Werth E, Radanov B, Ripley B, Nishino S, Mignot E. The narcoleptic borderland: a multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4:7–12.

38
Non-HLA Genes in Narcolepsy

MEHDI TAFTI
Center for Integrative Genomics, University of Lausanne, Lausanne-Dorigny, Switzerland

Since the first HLA association study in 1983, we know that narcolepsy has a genetic component and that HLA-DQB1*0602 confers an increased risk of the disease in all ethnic groups. However, no other gene has yet been unequivocally associated with narcolepsy. We also know that mutations in hypocretin/orexin ligands or receptors cause narcolepsy in animal models and that hypocretin/orexin deficiency characterizes human narcolepsy, although without any identified mutation in the hypocretin/orexin gene system. Narcolepsy is mainly sporadic, its clustering in families is rare, and the concordance rate in monozygotic twins is low. Therefore no convincing evidence for major non-HLA genetic factors is available. Thus, narcolepsy should be considered a complex disorder where many genes might be involved, each exerting a small effect. To fully understand the pathogenic mechanisms, a comprehensive knowledge of the effect of the individual genes and environmental factors is needed.

Narcolepsy has long been considered as a unique model of a primary disorder of vigilance states in otherwise healthy subjects. Therefore it has always been speculated that the discovery of its causes should shed light on the basic mechanisms of sleep regulation and ultimately sleep function(s). Owing to an animal model "canine narcolepsy," intensive research during the last 2 decades at Stanford Narcolepsy Center, brought invaluable information on neuroanatomy, neurochemistry, pharmacology, and genetics of narcolepsy. By linking mutations in hypocretin/orexin receptor 2 gene to canine narcolepsy (1) and subsequently discovering hypocretin/orexin deficiency as the hallmark of human narcolepsy (2,3), this unique example of a close interaction between basic animal and clinical research revolutionized our understanding of this complex disorder. However, as summarized below, the pathophysiology of human narcolepsy and its molecular substrates remain elusive.

As discussed in other chapters, the HLA association in narcolepsy discovered in 1983 (4) remains, to date, the only established genetic evidence. Because many HLA-associated disorders have an underlying immune/autoimmune abnormality, the autoimmune hypothesis of narcolepsy is regularly revisited without convincing evidence. Here, we will review the potential involvement of non-HLA genes in human narcolepsy and argue that even if hypocretin/orexin deficiency constitutes, beyond any doubt, the most specific characteristic of narcolepsy, hypocretin/orexin deficiency is sufficient but not necessary, and that the underlying cause might be complex.

I. Genetic Susceptibility to Narcolepsy in Families and Twins

Familial clustering of a disorder provides the first evidence for a genetic contribution to its etiology. If the pattern of inheritance follows an autosomal recessive or dominant transmission, a direct link between a genetic defect (single gene defect) and the phenotype is suspected and may lead to the positional cloning of a pathogenic gene mutation (as in the case of canine narcolepsy). Although different gene defects may explain a similar disorder in different families, the molecular cause remains relatively straightforward: a casual Genotype-Phenotype correlation. Narcolepsy may also be a simple genetic disorder because it is relatively rare and has a presumably "simple" cause: the hypocretin/orexin deficiency.

Westphal was the first author to report a family where the mother and one of the sons were affected with narcolepsy (5). In 1942, a family with 4 affected members was reported by Krabbe and Magnusse but the subjects seem also to be affected with the sleep apnea syndrome (6). The same authors reviewed the literature on 250 to 350 possible narcolepsy cases reported before 1942 and considered 54 of them to be familial. Daly and Yoss described a family with 13 affected members over 4 generations (7). Finally, Yoss and Daly have considered that one third of the 400 narcolepsy patients seen in their clinic could represent a family history of the same condition (8). Altogether, these authors have suggested an autosomal dominant mode of transmission with incomplete penetrance.

Thus till the 80s, narcolepsy was commonly considered to be familial in 25% to 50% of the cases. However, these percentages are not reliable because of variable diagnostic criteria. With recent and more stringent criteria, this rate varies between 6% and 18%. In a study including 334 Japanese narcolepsy patients, 6% presented a familial history of narcolepsy-cataplexy while 40% presented a familial history of excessive daytime sleepiness (EDS) (9). Segregation analysis in 419 Japanese families indicated that 3.45 % of the siblings were affected with full-blown narcolepsy-cataplexy and 10.34% were affected with EDS (10). The familial clustering of both conditions is largely higher than that found in the general Japanese population (0.16% and 0.52% for narcolepsy and EDS, respectively) and thus suggests a common genetic predisposition (10). Familial patterns of narcolepsy were also investigated in 334 unrelated cases seen at the Stanford Sleep Clinic before 1989 and in 6% of the cases family history of clear-cut narcolepsy and in up to 40% family history of EDS could be found (11). In this study the relative risk of narcolepsy in first-degree relatives was 6 to 18 times greater than that for unrelated individuals. In a French study including 188 narcolepsy patients, familial cases of narcolepsy-cataplexy represented 7.44% while 12.13% were represented with recurrent episodes of naps and/or lapses into sleep (RENLS) without cataplexy or any other sleep disorder (12). In this study, the frequency of narcolepsy was 1.6% among the parents and 0.63% among the siblings leading to a familial aggregation ratio of 1.06% whereas that of RENLS was 4.26% among the parents and 3.17% among the siblings leading to a familial aggregation ratio of 3.65%. The observed frequency of narcolepsy in first-degree relatives suggests an empirical risk for narcolepsy at least 40 times higher than that in the general population. Although no estimate of empirical risk for RENLS could be obtained due to the absence of prevalence data in the general population, again the familial clustering of narcolepsy and RENLS strongly suggests a common genetic predisposition. In a Czech study including 153 narcolepsy patients,

familial forms of narcolepsy-cataplexy represented 9.8% while 15% were represented with EDS (13). Among first-degree relatives, 1.2% were affected with narcolepsy-cataplexy and 4.28% with EDS, corroborating the general finding that both forms might share a common genetic basis. In a German study with 411 narcolepsy patients a family history of narcolepsy-cataplexy was present in only 1.4% while that of EDS was present in 7.2% (14). The relative risk for first-degree relatives to develop narcolepsy-cataplexy was 16.5 and 34.2 to develop EDS, again largely higher than the risk in the general population.

Based on the stringency of criteria for narcolepsy, authors reporting a high familial prevalence suggest an autosomal dominant mode of transmission while those reporting a lower familial prevalence suggest a polygenic or multifactorial mode of transmission. In any case, a trait transmitted as multifactorial with high heritability cannot be distinguished from a trait transmitted as purely autosomal dominant. Genetic heterogeneity has also been proposed since in some families the effect of a major gene is evident while in others polygenic or oligogenic effects can be observed.

The relative contribution of the genetic and environmental factors can be assessed in twins. In the case of simple disorders the concordance rate in monozygotic twins (probability of same phenotype in co-twins) is usually high (close to 100%), while in complex disorders the concordance rate is usually low (usually less than 50%). Therefore the concordance rate in monozygotic twins reflects a direct measure of respective contribution of genetic and environmental factors. Among the 16 monozygotic twin pairs described, only six are considered to be concordant (37.5%) and most of them are not completely documented. Interestingly, at least 2 concordant monozygotic twins are DQB1*0602 negative. A Japanese monozygotic twin pair was discordant for years but the unaffected co-twin developed a typical narcolepsy later at age 45, suggesting the presence of underlying genetic susceptibility factors that can lead to the disease once other factors (probably environmental) are present (15). One HLA-DQB1*0602 positive twin pair concordant for narcolepsy was recently reported with normal levels of CSF hypocretin-1 and without any mutation in the hypocretin genes system (16). This unique case of presumably genetic form of narcolepsy without hypocretin/orexin deficiency suggests that narcolepsy can occur with normal CSF hypocretin/orexin or in other words, that the genetic predisposition to narcolepsy may not be directly related to the availability of hypocretin/orexin peptides. More recently, we have reported a discordant HLA-DQB1*0602 positive monozygotic twin pair with undetectable CSF hypocretin/orexin in the affected and normal levels in unaffected co-twins (17). The presence of narcolepsy in this interesting twin pair does not seem to be genetic in nature.

Finally, in a small number of familial cases neither HLA-DQB1*0602 nor hypocretin/orexin deficiency segregate with narcolepsy indicating that narcolepsy is a complex disorder with substantial contribution of environmental factors.

II. Candidate Gene Approach in Narcolepsy

Based on the data summarized above a major conclusion is that with or without HLA-DQB1*0602 and hypocretin/orexin deficiency, other genetic susceptibility factors (and environmental factors) have to be involved. Obviously genes involved

in hypocretin/orexin neurotransmission are the best candidates. However, except for a single case, no pathogenic mutation has been found in either prepro-hypocretin/orexin or the two hypocretin/orexin receptor genes (including sporadic, familial, and HLA-DQB1*0602 negative cases of narcolepsy) (3). Thus even in the case of hypocretin/orexin deficiency, the underlying gene polymorphisms and/or gene defects remain unknown.

The candidate gene approach is based on available knowledge or hypothesis on pathophysiology of a disorder. Based on HLA association and pharmacology of narcolepsy two main gene groups constitute potential candidates: the immune/autoimmune (including apoptosis) and the neurotransmitter encoding genes.

Three studies reported higher tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) either in plasma or secreted by monocytes in narcolepsy patients, suggesting a role for proinflammatory cytokines (18–20). The TNF-alpha gene is located within the HLA region and is polymorphic. A higher level of TNF-alpha in narcolepsy may be due to a mutation in the regulatory region (promoter) of this gene. Thus, Kato et al. searched for such mutations in 92 narcolepsy patients and found a C to T conversion at position −850 but this polymorphism was not associated with narcolepsy when tested together with 91 normal subjects (21). The same group failed also to find any mutation in exons and introns of TNF-alpha, excluding the possibility of a pathogenic polymorphism or mutation associated with narcolepsy (22). Despite these negative results, another study searched for single nucleotide polymorphisms (SNPs) in the promoter region of TNF-alpha and found one at position −857, which was associated with narcolepsy although in a small (49 cases) population (23). The same group later reported a positive association between a presumably functional SNP (substitution between methionine and arginine at position 196) in the TNF-receptor 2 gene (TNFR2) and narcolepsy (24). They have proposed an additive effect of polymorphisms in both TNF-alpha and TNFR2 on the susceptibility to narcolepsy. The same group also analyzed TNF-alpha-HLA-DRB1*1501 haplotypes in 28 members of several narcolepsy families and discovered a rare predisposing haplotype (TNF-alpha(-857T)-HLA-DRB1*1501) in affected members (25). Finally, these results could be replicated in another study where narcolepsy patients were compared to DRB1*1501-DQB1*0602 positive control subjects, suggesting that TNF-alpha(-857T)-HLA-DRB1*1501-TNFR2-196R is a new susceptibility "haplotype" to narcolepsy (26). However, this finding seems specific to the Japanese population because a recent study in German narcolepsy patients found a strong association between TNF-alpha(-857T) and HLA-DRB1*15/16 negative but not in typical HLA-DRB1*15 positive narcolepsy patients (27). There was no association between HLA-DRB1*15 positive narcolepsy and either TNFR1 or TNFR2 (28). Also, the atypical HLA-DRB1*15 negative narcolepsy but not positive was found to be associated with a single nucleotide and a microsatellite polymorphisms of the alpha-interferon gene (29).

Extensive evidence from canine narcolepsy as well as from some human studies indicates that few neurotransmitter systems are critically involved in the control of narcolepsy symptoms. These include mainly noradrenergic, cholinergic, serotonergic, and dopaminergic systems. Based on abnormal REM sleep symptoms suggestive of narcolepsy in Norrie disease, Koch et al. studied several genetic markers in the Norrie disease region (on chromosome X) in 28 narcolepsy patients and found a positive association with a polymorphic marker in the monoamine oxidase-A (MAO-A) gene (30).

However, we have failed to confirm this finding in a large French narcolepsy population (31). We have also performed an association study between a functional polymorphism of catechol-O-methyltransferase (COMT) and narcolepsy and found no genotype or allele frequency differences as compared to a control population. Nevertheless, a sexual dimorphism and a strong effect of COMT genotype on narcolepsy severity were found (31). Women narcolepsy patients with high COMT activity genotype fell asleep (on multiple sleep latency test) twice as fast as those with low COMT activity genotype while the opposite was true for men. COMT genotype also strongly affected the presence of sleep paralysis and the number of REM sleep onset periods. In agreement with available pharmacological data, this study for the first time, provided genetic evidence for the involvement of the dopaminergic and/or noradrenergic rather than serotonergic systems in human narcolepsy. The implication of the COMT functional polymorphism in EDS is further demonstrated by the finding that the low activity COMT genotype is associated with a better response to stimulant Modafinil, especially in women narcolepsy patients (32). Two candidate serotonergic genes were also investigated for potential association with narcolepsy in a small (55 patients) German population (33). Neither polymorphism of 5-HT2A receptor nor that of tryptophan hydroxylase were associated with narcolepsy.

New techniques are becoming available to test for association between a phenotype and hundreds of candidate genes and pooled DNA is one of these techniques. Using a polled DNA technique, Wieczorek et al. screened 254 genes for association with narcolepsy in 100 narcolepsy patients and 100 control subjects (34). The candidate genes were from 3 gene groups: circadian, neurotransmission, and immunity-apoptosis. Few significant associations were found and included BAG1 (anti-apoptosis), COMT, dopamine receptor D2, GABA-B receptor 1, and serotonin receptor 2A. Note that COMT polymorphism was not previously found to be associated with narcolepsy in a French population (31). Finally, in relation to apoptosis and neurodegeneration, Gencik et al. performed an association study between narcolepsy and ApoE4 (associated with early-onset Alzheimer's disease) and found neither a significant association nor an age at onset effect (35). Overall, the only reliable finding remains the strong association with HLA-DQB1*0602.

III. Linkage Studies in Narcolepsy

A linkage study constitutes the first step in the localization and ultimate identification of a gene causally linked to a disease. However a major limitation is that large multiplex families are needed to have enough power to establish linkage between a chromosomal region and the disease. As narcolepsy is familial in less than 10% of the cases and families with multiple affected subjects over several generation are very rare, linkage studies are nearly impossible. Nevertheless, a first study was performed on 8 relatively small Japanese families where narcolepsy was associated with the typical HLA-DQB1*0602. Unfortunately, because of the lack of power, only a suggestive location was found on chromosome 4p13-q21 (36). This study has not been followed up for replication or candidate gene abnormalities in the identified region.

Familial forms of incomplete narcolepsy are more common than forms with full-blown narcolepsy (see above: Genetic susceptibility to narcolepsy in families and

twins). We have performed a genome-wide linkage analysis in a large French family with 4 (DQB1*0602 positive) members affected with narcolepsy-cataplexy and 10 others with isolated recurrent naps or lapses into sleep (the minor form of narcolepsy). A single significant location was identified on chromosome 21q (37). A single haplotype was shared by all affected individuals and informative crossovers indicated that the narcolepsy susceptibility gene in this family is located in a 5 Mb region of 21q. This region contains at least 40 potential genes amongst which some interesting candidates narcolepsy susceptibility genes such as inward rectifier potassium channels 6 and 15 (KCNJ6, KCNJ15), Purkinje cell protein 4 or brain-specific polypeptide 19 (PEP-19), Down syndrome cell adhesion molecule precursor (DSCAM), interferon-regulated resistance GTP-binding proteins A and B (MX1 and MX2), and a transmembrane serine protease (TMPRSS2). Because a potential involvement of PEP-19 is suspected in neurodegenerative disorders such as Alzheimer and Huntington's disease, we have sequenced this gene in 2 members of our family affected with full-blown narcolepsy but found no pathogenic mutation. Sequencing and expression profiling of the remaining genes in the region are underway to search for mutations and or polymorphisms that could segregate with narcolepsy. The identified region on chromosome 21 corresponds to the so-called "critical Down syndrome region," and a recent report on an atypical case of narcolepsy associated with Down syndrome (38) strengthens our finding that a narcolepsy susceptibility gene might be located on 21q.

IV. Conclusions

Although after the discovery of hypocretin/orexin involvement the cause of narcolepsy seemed simple, the genetic susceptibility factors remain elusive. Even if the hypocretin/orexin deficiency is the most specific biological correlate of narcolepsy, its cause (possibly autoimmune process) may turn out to be very complex and most probably not due to a single gene mutation. Obviously human narcolepsy is not a simple autosomal disorder and many genes and environmental factors might play a role in its variable expression and severity. Narcolepsy susceptibility genes are numerous, each having a small but still significant contribution. Future studies by including large narcolepsy populations from different ethnic origins and large number of candidate genes should have enough statistical power to identify even small effect genes. Genomewide linkage disequilibrium and microarray-based gene profiling would also be highly informative. Finally, more work is needed to better define the narcolepsy spectrum as well as the contribution of environmental triggering factors.

References

1. Lin L, Farac J, LiR, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–76.
2. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
3. Peyron C, Farac J, Rogers, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–7.

4. Honda Y, Asaka A, Tanaka Y, Juji T. Discrimination of narcolepsy by using genetic markers and HLA. Sleep Res 1983; 12:254.
5. Westphal C. Eigenthümliche mit Einschlafen verbundemne Anfälle. Arch Psychiatr Nervenkr 1877; 7:631–635.
6. Krabbe E, Magnussen G. On narcolepsy. I. Familial narcolepsy. Acta Psychiatr Neurol 1942; 17: 149–173.
7. Daly D, Yoss R. A family with narcolepsy. Proc Mayo Clin 1959; 34:313–319.
8. Yoss R, Daly D. Hereditary aspects of narcolepsy. Trans Am Neurol Assoc 1960; 85:239–240.
9. Honda Y, Asaka A, Tanimura M, Furusho T. A genetic study of narcolepsy and excessive daytime sleepiness in 308 families with a narcolepsy or hypersomnia proband. In: Guilleminault C, Lugaresi E, eds. Sleep/Wake Disorders: Natural History, Epidemiology and Long-Term Evolution. New York: Raven Press, 1983:187–199.
10. Honda Y. Clinical features of narcolepsy: Japanese experience. In: Honda Y, Juji T, eds. HLA in Narcolepsy. Berlin: Springer-Verlag, 1988:24–57.
11. Guilleminault C, Mignot E, Grumet FC. Familial patterns of narcolepsy. Lancet 1989; 2:1376–1379.
12. Billiard M, Pasquié-Magnetto V, Heckman M, et Carlander B, Besset A, Zachariev Z, Eliaou JF, Malafosse A . Family studies in narcolepsy. Sleep 1994; 17:S54-S59.
13. Nevsimalova S, Mignot E, Sonka K, Arrigoni JL. Familial aspects of narcolepsy-cataplexy in the Czech Republic. Sleep 1997; 20:1021–1026.
14. Mayer G, Lattermann A, Mueller-Eckhardt G, Svanborg E, Meier-Ewert K. Segregation of HLA genes in multicase narcolepsy families. J Sleep Res 1998; 7:127–133.
15. Honda M, Honda Y, Uchida S, Miyazaki S, Tokunaga K. Monozygotic twins incompletely concordant for narcolepsy. Biol Psychiatry 2001; 49:943–947.
16. Khatami R, Maret S, Werth E. Monozygotic twins concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 363:1199–1200.
17. Dauvilliers Y, Maret S, Bassetti, et al. A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology 2004; 62:2137–2138.
18. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997; 82:1313–1316.
19. Hinze-Selch D, Wetter TC, Zhang Y, et al. In vivo and in vitro immune variables in patients with narcolepsy and HLA-DR2 matched controls. Neurology 1998; 50:1149–1152.
20. Okun ML, Giese S, Lin L, Einen M, Mignot E, Coussons-Read ME. Exploring the cytokine and endocrine involvement in narcolepsy. Brain Behav Immun 2004; 18:326–332.
21. Kato T, Honda M, Kuwata S. Novel polymorphism in the promoter region of the tumor necrosis factor alpha gene: No association with narcolepsy. Am J Med Genet 1999; 88:301–304.
22. Kato T, Honda M, Kuwata S. A search for a mutation in the tumour necrosis factor-alpha gene in narcolepsy. Psychiatry Clin Neurosci 1999; 53:421–423.
23. Hohjoh H, Nakayama T, Ohashi J, et al. Significant association of a single nucleotide polymorphism in the tumor necrosis factor-alpha (TNF-alpha) gene promoter with human narcolepsy. Tissue Antigens 1999; 54:138–145.
24. Hohjoh H, Terada N, Kawashima M, Honda Y, Tokunaga K. Significant association of the tumor necrosis factor receptor 2 (TNFR2) gene with human narcolepsy. Tissue Antigens 2000; 56:446–8.
25. Hohjoh H, Terada N, Miki T, Honda Y, Tokunaga K. Haplotype analyses with the human leucocyte antigen and tumour necrosis factor-alpha genes in narcolepsy families. Psychiatry Clin Neurosci 2001; 55:37–39.
26. Hohjoh H, Terada N, Nakayama T, et al. Case-control study with narcoleptic patients and healthy controls who, like the patients, possess both HLA-DRB1*1501 and -DQB1*0602. Tissue Antigens 2001; 57:230–235.
27. Wieczorek S, Gencik M, Rujescu D, et al. TNFA promoter polymorphisms and narcolepsy. Tissue Antigens 2003; 61:437–442.
28. Wieczorek S, Dahmen N, Jagiello P, Epplen JT, Gencik M. Polymorphisms of the tumor necrosis factor receptors: no association with narcolepsy in German patients. J Mol Med 2003; 81:87–90.
29. Wieczorek S, Dahmen N, Kasten M, Epplen JT, Gencik M. A rare form of narcolepsy (HLA-DR2-) shows possible association with (functionally relevant) alpha-interferon gene polymorphisms. Psychiatr Genet 2004; 14:47–51.

30. Koch H, Craig I, Dahlitz M, Denney R, Parkes D. Analysis of the monoamine oxidase genes and the Norrie disease gene locus in narcolepsy. Lancet 1999; 353:645–646.
31. Dauvilliers Y, Neidhart E, Lecendreux M, Billiard M, Tafti M. MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 2001; 6:367–372.
32. Dauvilliers Y, Neidhart E, Billiard M, Tafti M. Sexual dimorphism of the catechol-O-methyltransferase gene in narcolepsy is associated with response to modafinil. Pharmacogenomics J 2002; 2:65–68.
33. Fehr C, Schleicher A, Szegedi A, et al. Serotonergic polymorphisms in patients suffering from alcoholism, anxiety disorders and narcolepsy. Prog Neuropsychopharmacol Biol Psychiatry 2001; 25:965–982.
34. Wieczorek S, Jagiello P, Arning L, Dahmen N, Epplen JT. Screening for candidate gene regions in narcolepsy using a microsatellite based approach and pooled DNA. J Mol Med 2004; 82:696–705.
35. Gencik M, Dahmen N, Wieczorek S, Kasten M, Gencikova A, Epplen JT. ApoE polymorphisms in narcolepsy. BMC Med Genet 2001; 2:9.
36. Nakayama J, Miura M, Honda M, Miki T, Honda Y, Arinami T. Linkage of human narcolepsy with HLA association to chromosome 4p13q21. Genomics 2000; 65:84–86.
37. Dauvilliers Y, Blouin JL, Neidhar E, et al. A narcolepsy susceptibility locus maps to a 5 Mb region of chromosome 21q. Ann Neurol 2004; 56:382–388.
38. Dominguez-Ortega L, Salin-Pascual RJ, Diaz-Gallego E. Narcolepsy-like symptoms in a patient with down syndrome and without obstructive sleep apnea. Sleep 2003; 26:285–286.

39
Mutation Screening of the Hypocretin System Genes in Narcolepsy

JULIETTE FARACO and EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

A strong familial component in narcolepsy was suggested in the original reports of narcolepsy by Westphal and Fisher in the 1870s (1). The proband described by Westphal had an affected mother, and the proband of Fisher had a sister affected by narcolepsy. We now know that narcolepsy most commonly occurs sporadically, rarely in families, and only 1% to 2% of first-degree relatives develop narcolepsy. Despite the low overall risk, first-degree relatives have a 10–40 times greater risk for the development of the disorder than general population. HLA antigen DQB1*0602 is the major susceptibility factor in the development of narcolepsy/cataplexy, as the majority of cases (90% to 100%) are found in association with this allele, in contrast to an allele frequency of 12% to 38% in the general population (1). However, the increased risk is not solely explained by co-inheritance of the HLA allele, thus other loci are likely to be involved in triggering the process or modulating the susceptibility conferred by HLA DQB1*0602. Other precipitating environmental factors are also thought to be required, as only 25% to 31% of monozygotic twins are concordant for narcolepsy, and due to the high prevalence of the DQ allele in the general population (1).

Among the rare cases of DQB1*0602-negative narcolepsy with definite cataplexy, CSF hypocretin-1 levels are more often within the normal range. Single genes with major influence on development of the disease (high penetrance) apart from HLA are likely to be involved in these cases: one third of non-DQ narcolepsy probands come from multiplex families, and concordant monozygotic twins are more frequently DQ negative. Multiplex families often have a mode of transmission that is suggestive of an autosomal dominant model (2).

Several large studies have focused on the hypocretin system genes as contributing loci in the pathogenesis of human narcolepsy. The three known hypocretin system genes are prepro-hypocretin (Hcrt) on chromosome 17q21, hypocretin receptor 1 (Hcrtr1) on chromosome 1p33, and hypocretin receptor 2 (Hcrtr2) on chromosome 6p11.

II. Studies Focused on DQ-Negative Subjects, Multiplex Families, and Proband-Parent Trios

The initial study by Peyron et al., focused on a highly selected group of narcolepsy patients, as the majority of narcolepsy cases are DQB1*0602-associated and sporadic, outright hypocretin gene mutations were considered unlikely. Thus, special emphasis was placed on rare non-DQ patients and probands from multiplex families where more highly penetrant loci were suspected to be involved (3). A total of 74 patients and 118 controls (all Caucasians) were studied, including 27 from multiplex families and 29 that were lacking HLA DQB1*0602. The study included two early onset DQB1*0602 negative patients (isolated cataplexy, onset 3 months; narcolepsy/cataplexy onset 6 months). The two coding exons of the hypocretin gene and the seven coding exons from each receptor were sequenced, together with at least 50 bp of flanking intron sequence, in order to identify coding alterations or mutations affecting mRNA splicing. Twenty eight DQB1*0602 positive sporadic subjects were also included to investigate possible allelic associations in the development of the disorder.

Fourteen polymorphisms were identified in the Hcrtr1 and Hcrtr2 loci, including at least 2 in each locus with relatively high allele frequencies (Hcrtr1: 111T/C; V408I; Hcrtr2: −25 A/C, 49 T/C; V308I, see Table 1). None of these polymorphisms were associated with or linked to narcolepsy and all were presumed benign. These findings excluded Hcrtr1 and Hcrtr2 as major contributors for genetic predisposition to human narcolepsy. A rare non-coding 5'UTR polymorphism in the preprohypocretin locus (−20 A) was also identified but could not be used to exclude associations of Hcrt alleles in narcolepsy. A common polymorphism was subsequently identified upstream of the Hcrt locus within an alu repeat (−909 C/T) (4). Association between the Hcrt locus and narcolepsy was examined using the Transmission Disequilibrium Test (TDT) in 105 trio families (proband (88% DQB1*0602-positive), and two parents). The TDT eliminates effects of population stratification that can complicate case control studies. There was no distortion of transmission of either allele at position −909, indicating that polymorphisms at the Hcrt locus are not typically associated with the development of narcolepsy.

Here, we also report new polymorphisms found among a small group of African American individuals; these were not reported in the original study of Peyron et al. (Table 1). African American individuals included two sporadic DQB1*0602 negative narcolepsy patients, four DQB1*0602-positive probands of multiplex narcolepsy families, and 56 controls. Seven previously described polymorphisms appear to be enriched, or only found in African Americans (Hcrt IVS +16 T; Hcrtr1 111 C, 836 A, IVS+6 T, 1222 A; Hcrtr2 942 G). In addition, we found five new polymorphisms only observed in African Americans. All of these appear to be benign and are not associated with the narcolepsy phenotype in families. This result illustrates the importance of matching and reporting specific ethnic groups in allele screening and association studies.

Hypocretin mutation screening has been performed in two monozygotic twin pairs. One MZ twin pair was concordant for narcolepsy with a very similar age of onset (7–9 years) and clinical presentation (5). Both were DQB1*0602-positive, but had normal CSF Hcrt-1 levels. The clinical concordance and normal Hcrt-1 levels were suggestive of a single gene defect, but sequencing of the three-hypocretin

system genes did not reveal abnormalities. The hypocretin genes were also sequenced in a discordant pair of DQB1*0602-positive MZ twins (6). The affected twin had undetectable CSF Hcrt-1, and no mutations were detected.

III. A Hypocretin Mutation in a Single Case of Narcolepsy

In the study by Peyron et al., one subject was found to have a mutation likely to cause narcolepsy (3). This case is unusual because of the very early onset of cataplexy at six months of age. The patient is HLA negative and has undetectable CSF hypocretin-1. Sleep Onset REM Periods during the MSLT, PLMs, and nocturnal bulimia were also reported. The mutation was not present in the unaffected mother but paternal genomic DNA was not available. To examine the functional consequences of this mutation, wildtype and mutant hypocretin sequences were transfected into neuroblastoma cells and expressed as fusion proteins with green fluorescent protein. The mutant peptide had diminished progression through the transgolgi network, with scant amounts present in mature secretory vesicles, demonstrating that the mutation results in abnormal trafficking of the peptide precursor. The mutant peptide accumulated in a branching tubular network, possibly consisting of smooth endoplasmic reticulum (costaining with syntaxin 17), an effect hypothetized to be toxic for the cell, leading to rapid neurodegeneration of hypocretin cells after birth.

IV. Hypocretin Screening in DQ-Positive Subjects

Three mutational screens have examined the role of the hypocretin loci in the development of narcolepsy, with a focus on DQB1*0602-associated cases [7,8,9]. The study by Olafsdottir et al., included 47 patients (67% with cataplexy, HLA status not described) and 75 controls. This study used a diverse ethnic pool from Iceland, the U.S. and France (56% Caucasian, 11% African American, 33% other ethnicity). Coding exons and splice junctions of the three genes were sequenced, and the common polymorphisms reported in the Peyron et al. study were all confirmed (7). Several additional polymorphisms, some of which were ethnic-specific, were also reported. None were associated with narcolepsy and all were presumed to be benign.

Gencik et al. screened the entire Hcrt gene using single-strand conformational analysis (SSCA) in 133 patients with narcolepsy (97% with cataplexy, HLA status not described in 127), and 189 controls. Ethnicity was not reported. Altered bands identified through SSCA were excised, re-amplified and sequenced. A rare polymorphism in the 5' untranslated region of the Hcrt gene (C3250T) was reported in 6 of 178 narcoleptic patients and in one out of 189 control subjects. The authors suggested a possible association between the T allele and the development of narcolepsy. This non-coding polymorphism is without clear functional relevance and is located within 2 bp of another rare, benign polymorphism (C-20A)[3]. This finding was not replicated in three other large studies (3,4,7). The polymorphism was not identified among 356 narcolepsy and 354 control chromosomes sequenced in other studies (Table 1). These results strongly suggest that the polymorphism is an ethnic-specific allele in this set of patients from Germany.

Table 1 Alleic Variance of HCrt, HCrtr1, and HCrt2 loc in Narcoleptic and Control Subjects

				Peyron et al. 2000[1], Hungs et al. 2001[4], Faraco 2004[6‡]		Faraco 2004[6]	
			Method	DNA sequencing[1,4,6] PCR-RFLP[4]		DNA sequencing	
				Caucasian		African American	
DNA change	refSNP ID	Domain	Amino acid change	Narcolepsy	Control	Narcolepsy	Control
Preprohypocretin (Hcrt)							
(−)909T → C[4]	rs760282	Alu repeat/ promoter	non-coding	0.764 (185)	0.73 (107)	n.t.	n.t.
(−)593A insertion[3]	rs5820451	promoter	non-coding	n.t.	n.t.	n.t.	n.t.
(−)71 A → C[6]		5′ UTR	non-coding	0.000 (62)	0.000 (15)	0.833 (6)	n.t.
(−)22C → T[3]		5′ UTR	non-coding	0.000 (115)	0.000 (92)	0.000 (6)	n.t.
(−)20C → A[1]	rs4796777	5′ UTR	non-coding	0.009 (115)	0.005 (92)	0.000 (6)	n.t.
IVS +16C → T[2]	rs990279	intron1	non-coding	0.000 (65)	0.000 (15)	0.500 (6)	n.t.
47T → G[1]		exon2 signal peptide	L16R	0.007 (72)	0.000 (106)	0.000 (6)	n.t.
Hypocretin receptor 1 (Hcrtr 1)							
(−)123C → G[2]		5′ UTR	non-coding	0.000 (68)	0.000 (40)	0.000 (4)	0.000 (10)
(−)70C → G[6]		5′ UTR	non-coding	0.000 (61)	0.000 (40)	0.125 (4)	0.009 (56)
68T → C[6]	rs11806980	exon 1 (N-ter. EC)	V23A	0.000 (60)	0.000 (40)	0.083 (6)	0.143 (56)
111T → C[1]	rs1056526	exon 1	synonymous	0.382 (68)	0.360 (40)	1.000 (4)	0.800 (10)
IVS +21G → T[2]		intron 1	non-coding	0.000 (68)	0.000 (40)	0.000 (4)	0.000 (10)
201C → T[2]		exon 2	synonymous	0.000 (68)	0.000 (15)	0.000 (3)	0.000 (10)
210C → T[2]		exon 2	synonymous	0.000 (68)	0.000 (15)	0.000 (3)	0.000 (10)
237G → A[2]		exon 2	synonymous	0.000 (68)	0.000 (15)	0.000 (3)	0.000 (10)
IVS + 40 G → A[6]		intron 2	non-coding	0.000 (61)	0.000 (15)	0.125 (4)	0.056 (9)
499G → A[2]		exon 3 (TMIV)	G167S	0.000 (68)	0.000 (15)	0.000 (3)	0.000 (10)
793C → A[1]		exon 5 (IL3)	L265M	0.007 (68)	0.000 (45)	0.000 (2)	0.000 (10)
836 G → A[2]**	rs7516785	exon 5 (IL3)	R279Q	0.000 (61)	0.000 (15)	0.250 (2)	0.053 (57)
842G → A[1]		exon 5 (IL3)	R281H	0.000 (68)	0.010 (45)	0.000 (2)	0.000 (10)
IVS +6C → T[1]		intron 6	non-coding	0.029 (68)	0.060 (40)	0.000 (3)	0.190 (8)
1222G → A[1]	rs2271933	exon 7 (C-ter. IC)	V408I	0.375 (68)	0.340 (46)	1.000 (4)	0.667 (9)
1307G → A[2]		3′UTR	non-coding	0.000 (68)	0.000 (46)	0.000 (4)	0.000 (10)
Hypocretin receptor 2 (Hcrtr2)							
28C → T[1]		exon 1 (N-ter. EC)	P10S	0.007 (70)	0.000 (90)	0.000 (6)	n.t.
31C → A[1]		exon1 (N-ter. EC)	P11T	0.014 (70)	0.006 (90)	0.000 (6)	n.t.
IVS -26A → C[1]*		intron 1	non coding	0.150 (67)	0.164 (58)	0.000 (5)	n.t.
IVS +49C → T[1]	rs9396073	intron 2	non coding	0.250 (70)	0.172 (61)	0.833 (6)	n.t.
IVS -4C → T[6]	rs9475219	intron 2	non coding	0.000 (62)	0.000 (14)	0.167 (6)	n.t.
577 T → A[1]		exon 3 (TMIV)	C193S	0.000 (70)	0.013 (39)	0.000 (6)	n.t.
846 G → A[2]	rs12111299	exon 5	synonymous	0.000 (70)	0.000 (35)	0.000 (4)	n.t.
853 C → A[2]		exon 5	synonymous	0.000 (70)	0.000 (35)	0.000 (4)	n.t.
877 G → A[2]		exon 5 (IL3)	I293V	0.000 (70)	0.000 (35)	0.000 (4)	n.t.
922G → A[1]	rs2653349	exon 5 (TMVI)	V308I	0.157 (70)	0.186 (35)	0.125 (4)	n.t.
942A → G[1]		exon 5	synonymous	0.014 (70)	0.014 (35)	0.125 (4)	n.t.
IVS -87 G → A[2]		Intron 6	non coding	n.t.	n.t.	n.t.	n.t.
1202C → T[1]		exon 7 (C-ter. IC)	T401I	0.007 (70)	0.000 (99)	0.000 (4)	n.t.

Footnotes on p. 440.

Olafsdottir et al., 2001[2]		Gencik et al., 2001[3], Thompson et al. 2004[5]		
DNA sequencing		SSCA followed by DNA Sequencing[3]		
Mixed		Not reported[3], mixed[5]		
Narcolepsy	Control	Narcolepsy	Controls	Comments
n.t.	n.t.			Benign polymorphism
n.t.	n.t.	0.004(133)	n.r.	Benign polymorphism
0.000 (57)‡	0.000 (85)‡	0.000 (133)‡	n.r.	Benign,only found in African Americans
0.000 (57)‡	0.000 (85)‡	0.017 (178)	0.002 (189)	Presumed benign polymorphism
0.000 (57)‡	0.000 (85)‡	0.000 (133)‡	n.r.	Presumed benign polymorphism
0.114 (57)	0.065 (85)	0.000 (133)‡	n.r.	Benign,likely only in African Americans
0.000 (57)‡	0.000 (85)‡	0.000 (133)‡	n.r.	Dominant mutation in early onset narcolepsy: delayed trafficking demonstrated in functional analysis
0.000 (48)	0.018 (83)			Benign polymorphism
0.000 (48)‡	0.000 (83)‡			Benign,only found in African Americans
0.000 (48)‡	0.000 (82)‡			Benign,only found in African Americans
0.480 (48)	0.520 (82)			Benign, enriched in African Americans
0.000 (47)	0.007 (75)			Benign polymorphism
0.009 (54)	0.000 (86)			Benign polymorphism
0.000 (56)	0.006 (86)			Benign polymorphism
0.000 (52)	0.006 (86)			Benign polymorphism
0.000 (52)‡	0.000 (86)‡			Benign,only found in African Americans
0.000 (55)	0.006 (88)			Benign polymorphism
0.000 (52)	0.000 (87)			presumed benign
0.019 (54)	0.011 (87)			Benign,only found in African Americans
0.010 (52)	0.006 (88)			Benign polymorphism
0.059 (51)	0.052 (86)			Benign, enriched in African Americans
0.577 (52)	0.559 (76)			Benign, enriched in African Americans
0.073 (48)	0.051 (78)			Benign polymorphism
0.000 (51)	0.006 (88)	0.000 (28)‡	0.000 (110)‡	Presumed benign[1,2] Identified in 0.007 (70)TS/ADHD[5:] mild functional impairment *in vitro*
0.020 (51)	0.000 (88)	0.000 (28)‡	0.000 (110)‡	Presumed benign; unlinked with familial narcolepsy phenotype[1] Identified in 0.036 (28) EDS[5:] mild functional impairment *in vitro*
0.202 (47)	0.187 (83)			Benign polymorphism
0.000 (51)‡	0.000 (88)‡			Benign polymorphism
0.000 (51)‡	0.000 (88)‡			Benign,likely only in African Americans
0.000 (51)‡	0.000 (88)‡			Benign polymorphism
0.020 (49)	0.006 (88)			Benign polymorphism, Tag SNP
0.000 (47)	0.006 (88)			Benign polymorphism
0.000 (49)	0.006 (88)			Benign polymorphism
0.108 (48)	0.154 (78)			Benign polymorphism
0.043 (46)	0.020 (76)			Benign, enriched in African Americans
0.000 (51)	0.024 (85)			Benign polymorphism
0.000 (51)‡	0.000 (88)			Possible weakly penetrant allele in combination with DQB1*0602[1]

(*Continued*)

Thompson et al. recently used SSCA to study the involvement of the Hcrtr2 gene in the etiology of narcolepsy (28 subjects, DQB1*0602-positive), excessive daytime sleepiness (EDS) (28 subjects, DQB1*0602-negative), Tourette syndrome with some EDS comorbidity (70 caucasian subjects, 57 meeting DSM IV), and in controls (110, ethnically-matched) (9). Two previously reported rare coding polymorphisms P10S and P11T (3,7) were observed in one Tourette patient with comorbid ADHD, and 2 EDS patients respectively, but not in the other groups. The P11T polymorphism was previously reported in one control subject, in two HLA negative narcolepsy/cataplexy multiplex family probands (3), and in 2 sporadic narcolepsy patients (7). The polymorphism did not co-segregate with narcolepsy in the family, was also identified among controls, and was presumed to be benign. The P10S polymorphism was identified in a single sporadic HLA-positive narcolepsy patient (3) and one control (7) and was also presumed to be benign. This hypothesis is reinforced by the lack of conservation of these residues in other mammalian species (9). Despite the lack of evidence for a significant effect, Thompson et al. measured calcium mobilization after ligand stimulation of receptors bearing the Hcrtr2 P10S and P11T polymorphisms in vitro. Both variants had slightly decreased calcium mobilization in response to high concentrations of ligand (P10S reached the level of significance) raising the possibility of a partial impairment in hypocretin transmission. It is not known whether this would be functionally significant under physiologic conditions, especially in heterozygous subjects. It is also unclear how mildly diminished function could cause a dominant phenotype in humans, when dogs, and mice that are heterozygous for null alleles at this locus, are unaffected.

V. Conclusion

In summary, narcolepsy is primarily a sporadic disorder with HLA DQB1*0602 acting as a common susceptibility allele. In rare cases, highly penetrant, non-HLA loci appear to be involved, but several studies (Table 1) have demonstrated that the hypocretin

---

Footnotes to Table 1.

Allele frequencies determined in each study are indicated with the number of patients in parentheses.

*Previously incorrectly reported as IVS-25A → C.

**Previously incorrectly reported as R279E.

‡ Frequency inferred from published data (polymorphism tested but not detected or not specifically reported).

†Data from Peyron, Hungs and Faraco studies were combined for this presentation, therefore narcolepsy subjects are enriched for rare DQ- and familial forms of the disorder. Studies not overlapping: Hcrt − 20, − 22 alleles not tested by Thompson; Hcrtr2 not studied by Gencik. Superscript numbers ([n]) indicate original publication describing each polymorphism. Polymorphisms identified in African American subjects and not previously reported are listed in italics. Nucleotide and amino acid (AA) changes are keyed to the initiation ATG codon. Protein domain is indicated for coding alterations.

Abbreviations: UTR, untranslated region; IVS, intervening sequence (intron) with position relative to adjacent exon; N-ter.EC, N-terminal extracellular domain; TM, transmembrane domain; IL, intracellular loop; C-ter. IC, C-terminal intracellular domain; n.t., not tested; n.r. not reported; TS/ADHD, Tourette syndrome comorbid with ADHD; EDS, excessive daytime sleepiness.

Source: From Refs. 3, 4, 10, 11.

ligand and receptor loci are not common contributors to the development of the disorder.

References

1. Mignot E. A Hundred years of narcolepsy research. Arch Ital Biol 2001; 139:207–220.
2. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
3. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R, Hungs M, Pedrazzoli M, Padigaru M, Kucherlapati M, Fan J, Maki R, Lammers GJ, Bouras C, Kucherlapati R, Nishino S, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
4. Hungs M, Fan J, Lin L, Lin X, Maki RA, Mignot E. Identification and functional analysis of mutations in the hypocretin (orexin) genes of narcoleptic canines. Genome Res 2001; 11:531–539.
5. Khatami R, Maret S, Werth E, Retey J, Schmid D, Maly F, Tafti M, Bassetti CL. Monozygotic twins concordant for narcolepsy-cataplexy without any detectable abnormality in the hypocretin (orexin) pathway. Lancet 2004; 63:1199–2004.
6. Dauvilliers Y, Maret S, Bassetti C, Carlander B, Billiard M, Touchon J, Tafti M. A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology 2004; 62:2137–2138.
7. Olafsdottir BR, Rye DB, Scammell TE, Matheson JK, Stefansson K, Gulcher JR. Polymorphisms in hypocretin/orexin pathway genes and narcolepsy. Neurology 2001; 57:1896–1899.
8. Gencik M, Dahmen N, Wieczorek S, Kasten M, Bierbrauer J, Anghelescu I, Szegedi A, Menezes Saecker AM, Epplen JT. A prepro-orexin gene polymorphism is associated with narcolepsy. Neurology 2001; 56:115–117.
9. Thompson MD, Comings DE, Abu-Ghazalah R, Jereseh Y, Lin L, Wade J, Sakurai T, Tokita S, Yoshida T, Tanaka H, Yanagisawa M, Burnham WM, Moldofsky H. Variants of the orexin2/hcrt2 receptor gene identified in patients with excessive daytime sleepiness and patients with Tourette's syndrome comorbidity. Am J Med Genet 2004; 129B:69–75.

40
Environmental Factors in Narcolepsy

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

CECILIA ORELLANA
Hospital de Clinicas, Instituto de Neurologia, Montevideo, Uruguay

I. Introduction

Narcolepsy is a disabling neurological disorder characterized by excessive daytime sleepiness and abnormal rapid eye movement (REM) sleep manifestations. A genetic predisposition with Human Leucocyte Antigen (HLA) DQB1*0602 has been evidenced as well as a strong association with absent or abnormally low cerebrospinal fluid (CSF) levels of the neuropeptide hypocretin. Several lines of evidence from family and twin studies are in favour of environmental factors in the development of narcolepsy. Family members of a narcoleptic subject may have the same haplotype as their relative and yet not be narcoleptics (Fig. 1). Also of 16 monozygotic twin pairs reported in the literature, five were presumably concordant and eleven discordant for narcolepsy (1). Moreover a recently reported HLA DQB1*0602 positive monozygotic twin discordant for narcolepsy was shown to be also discordant for CSF hypocretin-1 (2). This is clear evidence that the genetic background is not a sufficient condition to develop abnormality in the hypocretin system. Thus narcolepsy appears to result from the interaction of environmental factors acting on a specific genetic background. In a large study of adult-like sexed Finnish twin pairs it was shown that the best-fitting *a*dditive genetic components effects due to *d*ominance and nonshared *e*nvironmental components (ADE) model estimated that 34.7% of total variance was attributable to genetic effects among men and 39.4% among women, while over 65% was accounted for by environmental effects among men and 60% among women (3). In regard to these data it is striking that the literature should be so rich on genetic factors and so poor on environmental factors, probably due to the present aura of genetic research and the complexity and cost of relevant approaches to environmental factors. This is detrimental both in view of the understanding of the pathophysiology of narcolepsy and in view of its prevention. Thus we thought it valuable to review the present state of knowledge on environmental factors and to formulate proposals for the future. The issue of so-called symptomatic narcolepsies in relation with brain tumors, inherited disorders, vascular disorders, encephalopathies, multiple sclerosis, paraneoplastic syndromes is treated in another chapter (4) and will not be addressed.

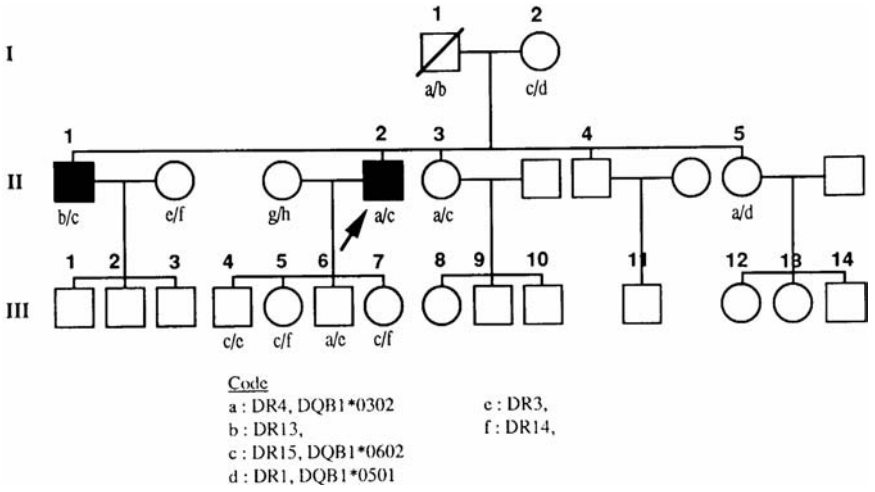


Figure 1 In a family with several HLA DR2/DQB1*0602 subjects, only two had narcolepsy with cataplexy, in favor of the role of environmental factors together with narcolepsy susceptibility to develop narcolepsy with cataplexy.

A. Clinical Data

There are scant reports of assumed environmental factors (5–6) in the development of narcolepsy. However it is not clear in these reports whether all subjects are true narcoleptic subjects. Of more interest, given the chronology of events and the laboratory tests performed, is the report of a 50-year-old Caucasian man who was stung by a non infectious fly (Tabanus sp.) very common in Quebec (7). This man developed in the following days a severe inflammatory reaction with a period of intense shivers without any fever probably triggered by the harmful character of the sting. Five days later, excessive daytime sleepiness, irresistible episodes of sleep and attacks of cataplexy occurred simultaneously. Multiple sleep latency tests documented five sleep onset REM episodes and HLA typing an association with HLA DR2.

Apart from these reports and others, a few systematic studies have been published documenting a variable proportion of patients reporting consistent life events in the day(s), week(s), or month(s) preceding the onset of the first symptom(s) of narcolepsy. In a series of 100 British narcoleptic subjects possible antecedents were given as the menarche in 4 subjects, pregnancy or child birth in 4, infectious disease in 2, emotional shock in 4, operation in 2, head injury in 1, and anaesthetic in 1 (8). In a series of 190 Japanese narcoleptic subjects, 17 (8.9%) reported clear or vague previous episodes of disturbed consciousness (after accident, operation, general anaesthesia, carbon monoxyde intoxication, high fever), 75 (39.4%) indicated preceding episodes, that might have been accompanied by disturbed consciousness, but intervals between the episodes and the onset of the illness were longer than three months, while 97 (51.0%) did not recall any episode of disturbed consciousness. However 7 (3.6%) of the latter reported extreme fatigue, overtime work, night shift work, sleep deprivation, etc., and 4 (2.1%) psychological stress including change of school, listening to a shocking war time story, sexual harassment, being informed of having syphilis (9). In our own series of

360 patients with narcolepsy with cataplexy, various circumstances were found, either a week or a month before the onset of the first symptom(s), in 40.2% of subjects, an ambiguous circumstance in 25.6% and no circumstance whatsoever in 34% (Table 1). The delay between the circumstance and the occurrence of the symptom(s) could be as short as a week or so. Several circumstances could act in a single subject (death of the mother, suicide of the father, going to boarding school, within a few months), and several circumstances too (divorce of the parents, moving home, financial difficulties) could affect two subjects in the same family, the mother aged 37 years and her son aged 15, starting narcolepsy at a few weeks' interval.

However, in none of these series were narcoleptic subjects compared with controls. Hence the value of a study in which 50 narcolepsy with cataplexy subjects and a control group of 50 individuals matched for sex, age, and socio-professional status (10), completed a life-event questionnaire, « the Schedule of Recent Experiences », a 40-item questionnaire pertaining to major areas of significance in the social structure (11). Narcoleptic subjects and controls were interviewed by the same investigator (C.O.), either in person or on the phone. Narcoleptic subjects were questioned about the existence of life events in the year(s) preceding the onset of excessive daytime sleepiness and the onset of cataplexy, and controls were questioned about the occurrence of life events in the corresponding year in which matched narcoleptic subjects developed the condition. The proportion of narcoleptic subjects reporting the presence of life-events in the year preceding the onset of excessive daytime sleepiness (82%) largely exceeded the proportion of controls (44%) ($p < 0.0001$) (Fig. 2) and the proportion of narcoleptic subjects reporting the presence of cataplexy in the year preceding the onset of cataplexy (84%) also largely exceeded the proportion of controls (36%)

Table 1 Suspected Environmental Factors at the Onset of Excessive Daytime Sleepiness and Cataplexy in a Population of 360 Subjects with Narcolepsy with Cataplexy (243 males and 117 females) Seen at the Sleep and Wake Disorders Center, Gui de Chauliac Hospital, Montpellier, France

Psychological stress	78 (21.6%)
Major emotional circumstance	19
Divorce, separation	18
Other familial turmoil	13
Major professional stress	13
Bereavement	11
War circumstances	4
Abrupt change of sleep/wakefulness rhythm and/or sleep deprivation	28 (7.7%)
Abrupt change of life rhythm	23 (6.3%)
Military service	11
Boarding school	7
Others	5
Pregnancy, infectious disease, head traumatism, etc.	16 (4.4%)
Ambiguous (unsettled interval between circumstance and onset of symptoms)	92 (25.5%)
None	123 (34.0%)

Note the large number of subjects in whom no obvious circumstance was found.

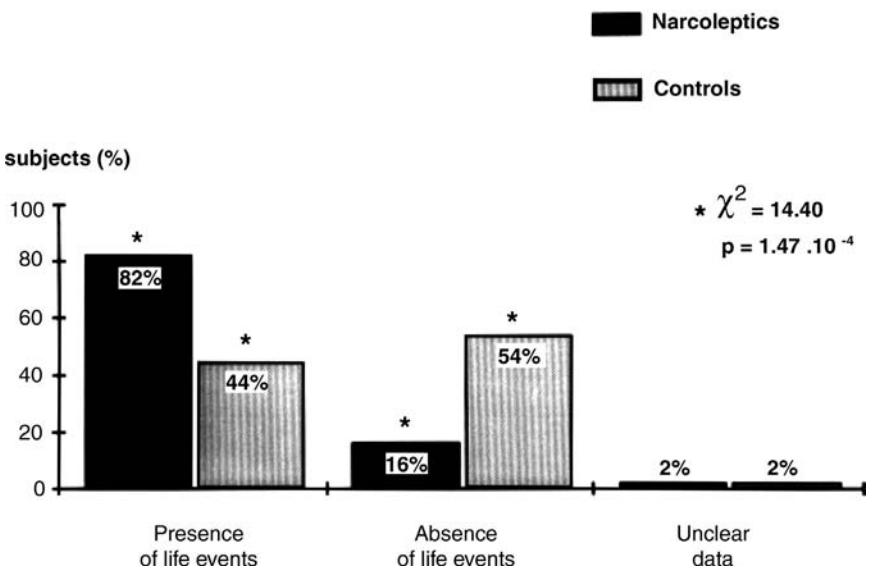


Figure 2 82% of narcolepsy subjects reported one or several life events in the year preceding the onset of excessive sleepiness, whereas only 44% of the control subjects referred to the existence of life events in the corresponding year ($p < 0.0001$). *Source*: From Ref. 10. Copyright 1994 by *Sleep*.

($p < 0.0001$). In addition the weight of life events proposed in « the Schedule of Recent Experiences » was evaluated by asking 50 individuals representative of the French population to give a weight to each item on a scale from 1 to 100, and the weight of life events reported by narcoleptic subjects was compared with that reported by control subjects. Remarkably, the weight of life events reported by narcoleptic subjects was significantly higher than the weight of those reported by control subjects in the corresponding years ($p < 0.001$). However some criticisms could be raised. The period of one year before the onset of the first symptom(s) was probably too long. Narcoleptic subjects may have had a better recollection of life-events in the year(s) preceding the onset of excessive daytime sleepiness and cataplexy, due precisely to the occurrence of these symptoms. Finally control subjects were matched for sex, age, and socio-professional status, not for HLA haplotype.

II. Focused Studies

A. Epidemiological Studies

The prevalence of narcolepsy is roughly the same (25 to 50 p 100.000 in all countries where surveys have been conducted (12), with the exception however of Israël, 0.23 p 100.000 (13) and Japan, 590 p 100.000 (14). However the methodology of some of these studies was somewhat questionable and the low frequency of HLA DQB1*0602 in the case of Israëli Jews (3.2%) (13) and its high proportion in the

case of Japanese (33.5%) (14) may play a role. A distinct interest of epidemiological studies in the search for environmental factors in a rare disease is the description of clusters of the disease that may raise the hope that local risk factors would be identified. We are not aware of such clusters in the case of narcolepsy, except for a debatable one, namely six narcoleptic subjects out of a population of 1385 participants in the Gulf War veterans, representing the catchment population of the Medical and Neurology Services, Detroit VA Medical Center (15). Out of 41 Persian Gulf War veterans evaluated for excessive daytime sleepiness and fatigue in the sleep center between January 1994 and December 1996, 6 of them (14.6%) had unequivocal narcolepsy-cataplexy equivalent to at least 0.43% of these 1385 individuals, that is about ten-fold the expected number based on the various estimates of prevalence in the literature. Special circumstances such as an abrupt change of sleep-wake schedule or a severe psychological stress, going to the war, preceded the occurrence of the first symptom(s) in some of these narcoleptics. However, as stated by the authors of this report, there were limitations due to referral and selection bias, lack of proper control group and the small sample size.

B. Infectious Agents

Infectious diseases preceding the onset of narcolepsy have long been reported. Wilson (5) described cases after influenza and encephalitis and Vein (6) after typhoid, pneumonia, mumps, scarlet fever, malaria, infectious hepatitis, and sinusitis. However the diagnosis of narcolepsy was questionable in several of these reports. More recently Parkes et al. (16) pointed out that in 15% of their narcoleptics the onset was sudden, sometimes after febrile and possibly viral illness, suggesting that narcolepsy may be « caught ». This prompted us to test systematically 52 narcoleptic subjects and 49 age and sex matched controls with a battery of 31 serological reactions corresponding to 20 bacterial and viral agents (17). Interestingly, elevated titers of serum antibodies to streptolysine 0 (ASO) and to streptodornase B (DNB) were evidenced, suggesting either a prolonged carrying of streptococcus possibly facilitated by a special immune background or a cross-reaction between some products of the streptococcus and a sleep substance, on the model of the proposed mechanism of poststreptococcus infection. However control subjects were not matched for HLA-DR antigens, and elevation of antibody titers was present in less than half of the patients. Nevertheless these results were confirmed in a group of 31 narcoleptic subjects compared with 18 subjects complaining of severe excessive daytime sleepiness without cataplexy, in which elevated titers of either ASO or DNB were also evidenced (18). On the other hand median values of ASO and DNB did not differ significantly in 100 narcoleptic subjects, 57 subjects with other hypersomnias and 107 healthy controls in another study (19).

C. Seasonality of Birth

Analysis of the seasonal pattern of birth can provide an indication on the environmental factors that may precipitate the occurrence of a disease, especially when the action of an infectious agent is thought as possible. Infectious disorders commonly show seasonal variations that may be reflected in the temporal pattern of birth of affected individuals. In the case of narcolepsy where an autoimmune process is suspected a birth seasonality in the occurrence of narcolepsy would strengthen this hypothesis. Preliminary data

based on a population of 484 narcoleptic subjects associating various ethnic groups (Caucasians, African Americans, Asians, Latinos, and subjects of mixed ethnicity) showed that subjects were born slightly more frequently during the month of March (20). More recently the birth dates of 866 patients with a diagnosis of narcolepsy with cataplexy, recruited in three different centers (Montpellier, France; Montreal, Canada; and Stanford, USA), were compared with those of 35 160 522 subjects from the general population (21). The monthly distribution of birth yielded a peak in March, with a maximum odds ratio at 1.45, and a trough in September, with a minimum odds ratio at 0.63. No gender or country of origin differences were observed. This seasonal predominance of birth suggests the role of early-life environmental factors, interacting with genetic susceptibility to cause damage to the hypocretin system.

D. Head Trauma

There have been a few reports of post-traumatic narcolepsy (8,22–23). However these reports were not consistent in the diagnostic criteria and polysomnography, multiple sleep latency test and HLA typing were often missing. That is to say the value of one center retrospective study dealing with 9 subjects, 6 females and 3 males, who had suffered mild to moderate closed head injury (24). All subjects had complaints of excessive daytime sleepiness and 5 subjects clearly demonstrated cataplexy. Important features were the mild to moderate character of head injury, the location of the head injury variable from patient to patient, the notion of some degree of loss of consciousness in 8 of the 9 patients, the normal neurologic evaluation and brain imaging (computerized tomography scan or magnetic resonance imaging), and the presence of one SOREMP in one subject and of two or more in the eight other subjects documenting narcolepsy. These data show that head traumatism may lead to narcolepsy, but the most interesting finding is the mild to moderate character of head traumatism relating narcolepsy more likely to a stress than to a neurologic damage.

III. Mechanisms

Narcolepsy is known to be associated with HLA DR2 – DQB1*0602. Since association with HLA is a hallmark of most autoimmune diseases, it has been proposed that auto-immunity may play a role in the development of narcolepsy. Moreover the complex genetic inheritance of human narcolepsy, its prepubertal onset and the biphasic profile of its age of onset speak in favour of auto-immunity. However, no inflammatory process has been demonstrated up to now, nor have the systemic immune abnormalities usually found in autoimmune pathologies been detected (25). More recently, a loss of hypocretin neurons has been evidenced in the brain of narcoleptic subjects (26). These neurons are located in the lateral hypothalamus and project widely through the brain (27). The absence of hypocretin neurons may indicate either a lack of transcription in intact cells or a previous destruction of hypocretin containing neurons targeted by an autoimmune process (25). If the hypothesis of an autoimmune mechanism is tentatively held true, its mechanism could include the role of unspecific stressors acting on a background of genetic susceptibility through the hypothalamic-pituitary-adrenal (HPA)

and the sympathetic-adrenal-medullary (SAM) axes, to result into the destruction of hypocretin neurons. Additionally, alteration of the cytokine network may affect neurodevelopment in utero or early in life, neuronal survival and complex brain functions.

In addition to the role of environmental factors in triggering narcolepsy, it is also of importance to mention the role of psychological factors as modulators of narcoleptic symptoms. This fact is best exemplified by the different evolution of narcoleptic symptoms in concordant twin pairs, in which one of the twins experienced exacerbation of her symptoms by the turmoil in her marriage while the other twin experienced improvement when remarriage brought emotional and financial security (28–29).

IV. For the Future

There are very few solid studies on the environmental factors involved in the development of narcolepsy. In order to strengthen clinical data it would be advisable to develop structured questionnaires aimed at collecting reliable data on demography, medical and family history, psychological profiles, life events exposure both in subjects with typical narcolepsy with cataplexy and in age, sex, ethnicity, socio-economic level, HLA DR2 / DQB1*0602 haplotype matched controls.

From an epidemiological standpoint it would be worth comparing the incidence of narcolepsy with cataplexy in population born, grown up and settled in one country and in population of the same ethnicity having emigrated to a distant country, like Indians living in India and Indians living in UK, Maghrebian Arabs living in North Africa and Maghrebian Arabs living in France, Latin-Americans living in Peru, Ecuador, or Bolivia and Latin Americans living in Spain.

The infectious issue should be further explored in comparing subjects with narcolepsy with cataplexy, with HLA matched normal controls.

V. Conclusion

We are certainly still far from defining with precision the factors or the groups of factors that may trigger narcolepsy in a genetically liable subject, and even further from disentangling the mechanisms by which these factors may act on the central nervous system to product narcolepsy. However the role of environmental factors cannot be ignored. Some suggestions have been made in view of making further steps in this domain.

References

1. Mignot E. Genetic and familial aspects of narcolepsy. Neurology 1998; 50(Suppl 1):S16–S22.
2. Dauvilliers Y, Maret S, Bassetti C, Carlander B, Billiard M, Touchon J, Tafti M. A monozygotic twin pair discordant for narcolepsy and CSF hypocretin-1. Neurology 2004; 62:2137–2138.
3. Kaprio J, Hublin C, Partinen M, Heikkila K, Koskenvuo M. Narcolepsy-like symptoms among adult twins. J Sleep Res 1996;5:55–60.
4. Kanbayashi T, Abe M, Mishikawa Y, Shimuzu T, Nishino S. Symptomatic narcolepsy with cataplexy and without cataplexy or hypersomnia, with and without hypocretin (orexin) deficiency. In: Bassetti C, Billiard M, Mignot E, eds. Narcolepsy and Hypersomnia. Informa, 307.
5. Wilson SAK. The narcolepsies, Brain 1928; 51:63–109.

6. Vein AM. Syndrome of hypersomnia (Narcolepsy and other forms of pathological sleepiness), Moscow: Izdatelstvo Meditsina 1966.
7. Montplaisir J, Poirier G, Godbout R, Marinier R. La narcolepsie: un modèle d'étiologie multifactorielle. Médecine/Sciences 1988; 4:239–244.
8. Parkes JD. Sleep and its disorders. W.B. Saunders Company, London 1985.
9. Matsuki K, Honda Y, Satake M, Juji T. HLA in narcolepsy in Japan. In: Y. Honda, T. Juji (eds) HLA in Narcolepsy. Springer Verlag, Berlin 1988:58–75.
10. Orellana C, Villemin E, Tafti M, Carlander B, Besset A, Billiard M. Life events in the year preceding the onset of narcolepsy. Sleep 1994; 17:S50–S53.
11. Rahe RH, Holmes TH. Social stress and illness onset. J Psychosom Res 1964; 8:35–44.
12. Ohayon M. Epidemiology of narcolepsy. In: Narcolepsy and Other Hypersomnias of Central Origin. Bassetti C, Billiard M, Mignot E (eds). Informa, this volume, p. 125.
13. Wilner A, Steinman L, Lavie P, Peled R, Friedmann A, Brautbar C. Narcolepsy-cataplexy in Israeli Jews is associated exclusively with the HLA DR2 haplotype. Hum Immunol 1988; 21:15–22.
14. Tashiro T, Kanbayashi T, Iijima S, Hishikawa Y. An epidemiological study on prevalence of narcolepsy in Japanese. J Sleep Res 1992; 1 (suppl 1):228.
15. Elhalwagi BM, Milnner IB, Koshorek G, Burchart C, Kapen S. Sleep disorders in Persian Gulf War veterans. Sleep Res 1997; 26:359.
16. Parkes JD, Langdon N, Lock C. Narcolepsy and immunity. Brit Med J 1986; 292:359–360.
17. Billiard M, Laaberki MF, Reygrobellet C, Seignalet J, Brissaud L, Besset A, Elevated antibodies to streptococcal antigens in narcoleptic subjects. Sleep Res 1989; 18:201.
18. Montplaisir J, Poirier G, Lapierre O, Montplaisir S. Streptococcal antibodies in narcolepsy and idiopathic hypersomnia. Sleep Res 1989; 18:271.
19. Mueller-Eckhardt G, Meier-Ewart K, Schiefer HG. Is there an infectious origin of narcolepsy? The Lancet 1990; 335:424.
20. Okun ML, Lin L, Pelin Z, Hong S, Mignot E. Clinical aspects of narcolepsy-cataplexy across ethnic groups. Sleep 2002; 25:27–35.
21. Dauvilliers Y, Carlander B, Molinari N, Desautels A, Okun M, Tafti M, Montplaisir J, Mignot E, Billiard M. Month of birth as a risk factor for narcolepsy. Sleep 2003; 26:1–3.
22. Good JL, Barry E, Fishman PS. Posttraumatic narcolepsy: the complete syndrome with tissue typing. J Neurosurgery 1989; 71:765–767.
23. Maccario M, Ruggles KH, Meriwether MW. Post-traumatic narcolepsy. Military Med 1987; 152: 370–371.
24. Lankford DA, Wellman JJ, O'Hara C. Post-traumatic narcolepsy in mild to moderate closed head injury. Sleep 1994; 17:S25–S28.
25. Mignot E, Tafti M, Dement WC, Grumet FC. Narcolepsy and immunity. Advances in Neuroimmunology 1995; 5:23–37.
26. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6:991–997.
27. Peyron C, Tighe DK, Van der Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kimlduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18:9996–10015.
28. Mamelak M, Caruso VJ, Stewart K. Narcolepsy: a family study. Biol Psychiatry 1979; 14:821–834.
29. Honda M, Honda Y, Uchida S, Miyazaki S, Tokunaga K. Monozygotic twins incompletely concordant for narcolepsy. Biol Psychiatry 2001; 49:943–947.

41
Autoimmune Studies in Narcolepsy

MICHAEL H. SILBER
Department of Neurology and Sleep Disorders Center, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

JOHN L. BLACK
Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Rochester, Minnesota, U.S.A.

LOIS E. KRAHN
Department of Psychiatry and Psychology, Mayo Clinic College of Medicine, Scottsdale, Arizona, U.S.A.

PAUL A. FREDRICKSON
Department of Psychiatry and Psychology and Sleep Disorders Center, Mayo Clinic College of Medicine, Jacksonville, Florida, U.S.A.

The extremely close association of HLA DR2 to narcolepsy with cataplexy was first noted in 1984 (1). Later work showed that the disorder was actually associated with HLA DQB1*0602 (2) and that the HLA gene itself, rather than another in close linkage disequilibrium, was the true marker of the disorder (3). Most cases of human narcolepsy are sporadic and monozygotic twins are usually discordant for the disease (4). Narcolepsy tends to start relatively abruptly, most often in the 2nd decade of life (5). These considerations have resulted in the formulation of an autoimmune hypothesis to explain the pathogenesis of human sporadic narcolepsy. This paper will summarize the available data.

I. Studies of Humoral Immunity (Excluding the Hypocretin System)

Based on the premise that multiple autoimmune disorders often coexist in the same patient, investigators have searched for evidence of serum autoantibodies to a range of antigens in patients with narcolepsy. Enzyme linked immunosorbent assays were used to test for rheumatoid factor and serum anti-DNA, anti-histone, anti-nuclear, anti-smooth muscle and anti-Sjogren syndrome B antibodies in 11 patients with narcolepsy and 10 with obstructive sleep apnea (OSA) syndrome (6). Eight positive tests were found out of 66 in the narcolepsy group compared to 9 positives out of 60 tests in the OSA group.

Serum neuron-specific and non neuron-specific antibodies were assessed in 43 patients with narcolepsy (7), all with cataplexy, sleep onset REM periods (SOREMP) on a multiple sleep latency test (MSLT), or both. Cataplexy was present in 33 patients and 30 were HLA DQB1*0602 positive. Neuron-specific antibodies

tested were N and P/Q type voltage gated calcium channel, nicotinic acetylcholine, Purkinje cell (anti-Yo), antineuronal nuclear, type 1 (anti-Hu), antineuronal nuclear, type 2 (anti-Ri) and amphiphysin antibodies. Non-neuron-specific antibodies tested were antinuclear, antimitochondrial, anti-smooth muscle, glutamic acid decarboxylase 65, thyroid microsomal (peroxidase) and thyroglobulin antibodies. Thirty percent of the patients had 1 or more antibodies present but no single antibody predominated. The frequency of positive studies was no more frequent than would be predicted in performing 12 tests on each patient. The presence of antibodies was not associated with cataplexy or HLA DQB1*0602 positivity.

Serum antibodies to streptococcal antigens have been assessed in a number of studies. In one study, elevated antibodies to streptolysin O (ASO), DNAse B (DNB) or both were found in 52% of 52 narcoleptic patients compared to 2% of 49 controls (8). Another study confirmed the high frequency of positive antibodies in a sample of 31 narcoleptics with cataplexy and SOREMP on a MSLT, but found a similar frequency of antibodies in a control group of 18 sleepy patients without cataplexy (9). Similar high frequencies of antibodies to ASO or DNB was found in a study of 100 patients with narcolepsy and cataplexy, 57 patients with other forms of hypersomnia but also in 107 healthy control subjects (10).

Low CSF hypocretin-1 levels have been found in occasional patients with Guillain-Barre syndrome (GBS). Autoantibodies in this disorder are frequently directed against gangliosides and thus a range of anti-ganglioside antibodies was assessed in 28 patients with narcolepsy, 26 of whom had cataplexy patients were positive for HLA DQB1*0602 and had CSF hypocretin-1 (hcrt-1) levels <100 pg/ml (11). Low titers of IgM antibodies were found in 15% of patients, a similar percentage to that reported in healthy controls. A single patient with a past history of the Miller-Fisher variant of GBS had anti-GQ1 antibodies, which are known to be associated with this disorder. Anti-ganglioside antibodies were not found in the CSF.

Anti-Ma2 antibodies are found in a rare form of paraneoplastic encephalitis usually associated with germ-cell tumors of the testis. The disorder affects the limbic system, hypothalamus and brainstem and may manifest with excessive daytime sleepiness. CSF Hcrt-1 concentration was <100 pg/ml in 4 of 6 patients with anti-Ma2 associated encephalitis, leading the investigators to test for serum and CSF anti-Ma2 antibodies in 19 patients with narcolepsy (12). No anti-Ma2 antibodies were found. Nevertheless, anti-Ma2 associated encephalitis provides a model of an autoimmune disorder affecting the hypocretin system.

Non-specific markers of immune mediated inflammation have been sought in the CSF of narcoleptic patients. Two of 15 patients with narcolepsy associated with 2 or more SOREMPS on MSLT had CSF oligoclonal bands and 1 of these had an increased CSF IgG index. The CSF white blood cell count was normal in all patients (13). These findings were similar to those seen in non-inflammatory neurological disorders in the investigators' laboratory.

Serum antibodies directed against neurons and brain tissue were assessed in 11 patients with narcolepsy compared to 10 patients with OSA (6). Equal numbers of patients and controls were found to have anti-neuronal antibodies to neuroblastoma cells. Immunofluorescence studies searching for antibodies to unfixed rat brain sections

were negative, as were immunofluorescence studies for antibodies against fractionated primate brainstem.

Purified serum IgG was obtained from 9 patients HLA DQB1*060: positive patients with narcolepsy. In a passive transfer experiment, the IgG enhanced the contractile response of mouse bladder detrusor muscle to cholinergic stimulation compared to control IgG (14).

In summary, no definite evidence has been found for a propensity to humoral-mediated autoimmune disease in patients with narcolepsy and searches for antibodies to a few candidate antigens not involving the hypocretin system have been negative. Although these results do not provide evidence in support of an autoimmune hypothesis for narcolepsy, they do not rule it out. Most studies involved serum rather than CSF and tested for antibodies against antigens unlikely to be directly involved in the pathogenesis of the disorder. The identification of a putative functional antibody affecting cholinergic stimulation of smooth muscle is intriguing but needs further confirmation.

II. Studies of Cell Mediated Immunity and Cytokine Levels

Limited studies of cellular immunity in narcolepsy have been reported. Nine patients with sleepiness, cataplexy and 2 or more SOREMPs on MSLT, all of whom were HLA DR2 positive, were compared to 9 DR2 positive and 9 DR2 negative controls (15). T-lymphocyte subsets were similar in all groups. No differences were noted in mitogen stimulated lymphocyte proliferation and natural killer activity. In a study of 31 narcoleptic patients compared to 55 controls, no differences were found in lymphocyte subsets, including 2 patients studied within 3 months of onset of the disorder (16). Restimulation rates of primed lymphocytes against DR2 haplotypes of narcolepsy patients were studied (17). The rates triggered by the cells of narcolepsy patients and controls were not different.

Plasma tumor necrosis factor alpha (TNF-α) and interleukin-6 were increased in 39 patients with narcolepsy (38 DQBB1*0602 positive) compared to 40 controls (18). However, similar increases in TNF-α have been found in patients with OSA (19), suggesting that an increase in inflammatory cytokines may be a non-specific consequence of excessive sleepiness rather than a marker of immune dysfunction. In contrast, plasma levels of TNF-α, IL-6 and interleukin 1ra were reported to be normal in 9 patients with narcolepsy and cataplexy compared to 18 controls (15). IL-6 secretion by peripheral monocytes after stimulation by lipopolysaccharides was higher in narcoleptics compared to HLA DR2 positive controls, but not after stimulation with phytohemagglutinine. There were no differences in the secretion of TNF-α, interleukins 1ra or 1β.

In summary, studies of peripheral cell mediated immunity and cytokine production do not provide definite evidence in support of an autoimmune hypothesis of narcolepsy.

III. Studies of the Hypocretin System

Our laboratory initially tested the hypothesis that patients with narcolepsy have serum antibodies specific for preprohypocretin and its derivatives by testing sera

mnlpstkvswaavtlllllllppallssgaaaqPLPDCCRQKTCSCRLYELLHGAGNHAAGILTLQ
KRRSGPPGLQGRLQRLLQASGNHAAGILTM*GRR(AGAEP[APRPCLGRR***CSAPAA**
A]SVAPGGQSGI

Figure 1 Preprohypocretin polypeptides used in ELISA testing of narcoleptic subjects. Lowercase letters are the N-terminal peptide, which is cleaved off during posttranscriptional processing. *Underlined* letters are hypocretin 1, letters underlined by a *dashed line* are the linker between hypocretin 1 and 2 sequences, *double underlined* letters are hypocretin 2, italicized letters are test peptide 1, bold letters are test peptide 2, *bracketed* letters are test peptide 3 and letters enclosed in *parenthesis* are test peptide 4.

of 34 strictly diagnosed HLA DQB1*0602-positive narcoleptic patients with cataplexy for evidence of autoantibodies against human preprohypocretin, hypocretin 1 and 2, N-terminal leader and C-terminal peptides of preprohypocretin using enzyme-linked immunosorbent assays (ELISA) (see Figure 1 for the antigens tested). These results were compared to 49 non-narcoleptic psychiatric and sleep apnea controls. ELISA measurements were in optical density (OD). Primary analyses were of the entire narcoleptic and control groups for each potential antigen, and none of the differences reached P values that were significant after Bonferroni adjustment. (20). Methodological issue specific to ELISA and the fact that we did not test CSF may have lead to false negative results in that study so the research was extended to serum studies of 41 DQB1*0602-positive narcoleptic subjects with cataplexy and 55 controls and CSF studies of 19 individuals with narcolepsy and 13 controls. All subjects who donated CSF had CSF hypocretin 1 levels < 40 pg/mL except for one patient who was 59 pg/mL (mean control hypocretin 1 level 352.8 ng/mL). We tested for IgG reactive to preprohyocretin and its major cleavage products (including hypocretin 1 and 2), using immunoprecipitation assays (IP), immunofluorescence microscopy of Chinese hamster ovarian cells expressing preprohyocretin, and Western blots. There was no evidence for IgG reactive to preprohyocretin or its cleavage products in the CSF of subjects with narcolepsy as measured by these tests. Although the IP with CSF and the C-terminal peptide showed significant differences by two methods of comparison, the control subjects had higher counts per minute than narcoleptic subjects, which was opposite the hypothesis (21).

We further tested that hypothesis that IgG in the cerebrospinal fluid of HLA DQB1*0602 positive narcoleptic subjects with cataplexy binds to rat hypothalamus protein extract as detected by ELISA. Forty five narcoleptic subject with cataplexy provided serum and were compared to 57 non-narcoleptic controls. Twenty patients with narcolepsy and 20 control subjects also provided CSF samples. Significant differences were detected when the narcoleptic and control subjects' CSF were compared at doubling dilutions ranging from 1:2 to 1:64. Significance remained at the 1:2, 1:4, and 1:8 dilutions even when the 6 highest narcoleptic OD scores were treated as outliers and removed from the analysis. No significant differences were observed at any titer when comparing the narcoleptic and control subject serum samples for the entire group, possibly due to high observed background in the serum ELISA assay. To our knowledge, this is the first evidence that narcoleptic subjects' CSF contains IgG that binds to hypothalamic protein (22). Additionally, we obtained similar results and significance when we repeated the test using baboon hypothalamus extract. High degrees

of significance were observed by ELISA in doubling dilutions ranging from 1:4 to 1:64 (unpublished data).

In summary, our studies have not provided evidence in support of an autoimmune reaction to any components of the hypocretin system in narcolepsy. However, there is preliminary evidence for the presence of one or more antigens in the CSF of patients with narcolepsy binding to hypothalamic proteins of as yet undetermined nature.

IV. Narcolepsy in Other Autoimmune Diseases

Cases of narcolepsy associated with hypopthalamic or pituitary pathology have been reported (23). While many of these have been due to neoplasms, occasional cases have been ascribed to disorders with a perceived inflammatory or autoimmune pathogenesis. These include patients with sarcoidosis (23,24) and acute disseminated encephalomyelitis (ADEM). Two cases of ADEM with sleepiness (but no cataplexy), hypothalamic abnormalities on MRI scans, and low CSF Hcrt-1 levels have been reported (25,26) Occasional cases of narcolepsy developing after the onset of multiple sclerosis have been reported (27,28). While these cases do not prove that idiopathic narcolepsy is of autoimmune origin, they do provide examples of how secondary narcolepsy can conceivably be caused by autoimmune disorders.

V. Pathology Studies

Limited studies of the pathology of human narcolepsy are available. A study of 4 narcoleptic brains compared to 12 controls showed an 85–95% reduction in the number of hypocretin synthesizing cells in the hypothalamus with increased gliosis (29). In contrast, another study of 2 narcoleptic brains failed to demonstrate hypothalamic cell loss or gliosis (30). In situ hybridization with TNF-α and HLA DR immunocytochemistry did not reveal evidence for persistent inflammation, although both cases were examined more than 50 years after disease onset.

VI. Future Directions

The hypothesis that human narcolepsy is an auto-immune disease remains extremely attractive. While current data does not provide strong support for the concept, further work is needed to investigate abnormal immune responses to different hypothalamic antigens.

Acknowledgments

We acknowledge the technical help of Rajeswari K. Avula and Denise L. Walker and the assistance of our research coordinator, Nancy L. Slocumb. Supported by NIMH MH 62599, by the Mayo Clinic Small Grants Fund, by the Ruan Family Grant for Proteomic Research to the Mayo Clinic, and by the Piscopo Funds of the Mayo Foundation.

References

1. Juji T, Satake M, Honda Y, Doi Y. HLA antigens in Japanese patients with narcolepsy: all the patients were DR2 positive. Tissue Antigens 1984; 24:316–319.
2. Mignot E, Lin X, Arrigoni J, Macaubas C, Olive F, Hallmayer J, Underhill P, Guilleminault C, Dement WC, Grumet PC. DQB1*0602 and DQA1*0102 (DQ1) are better markers than DR2 for narcolepsy in caucasian and black Americans. Sleep 1994; 17:S60–S67.
3. Ellis MC, Hetisimer AH, Ruddy DA, Hansen SL, Kronmal GS, McClelland E, Quintana L, Drayna DT, Aldrich MS, Mignot E. HLA class II haplotype and sequence analysis support a role for DQ in narcolepsy. Immunogenetics 1997; 46:410–417.
4. Mignot E. Genetic and familial aspects of narcolepsy. Neurology 1998; 50:S16–S22.
5. Silber MH, Krahn LE, Olson EJ, Pankratz VS. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population based study. Sleep 2002; 25:197–202.
6. Rubin RL, Hajdukovich RM, Mitler MM. HLA-DR2 association with excessive somnolence in narcolepsy does not generalize to sleep apnea and is not accompanied by systemic autoimmune abnormalities. Clin Immunol Immunopathol 1988; 49:149–158.
7. Black JL, Krahn LE, Pankratz VS, Silber MH. Search for neuronal-specific and non-neuronally-specific antibodies in the sera of patients with HLA DQB1*0602 positive and negative narcolepsy. Sleep 2002; 25:719–723.
8. Billiard M, Laaberki MF, Reygrobellet C, Seignalet J, Brissaud L, Besset A. Elevated antibodies to streptococcal antigens in narcoleptic subjects (abstr). Sleep Res 1989; 18:201.
9. Montplaisir J, Poirier G, Lapierre O, Montplaisir S. Streptococcal antigens in narcolepsy and idiopathic hypersomnia (abstr). Sleep Res 1989; 18:271.
10. Mueller-Eckhardt G, Meier-Ewart K, Schiefer HG. Is there an infectious origin of narcolepsy? Lancet 1990; 335:424.
11. Overeem S, Geleijns K, Garssen MPJ, Jacobs BC, van Doorn PA, Lammers GJ. Screening for anti-ganglioside antibodies in hypocretin-deficient human narcolepsy. Neurosci Lett 2003; 341:13–16.
12. Overeem S, Dalmau J, Bataller L, Nishino S, Mignpot E, Verschuuren J, Lammers GJ. Hypocretin-1 CSF levels in anti-Ma2 associated encephalitis. Neurology 2004; 62:138–140.
13. Fredrikson S, Carlander B, Billiard M, Link H. CSF immune variables in patients with narcolepsy. Acta Neurol Scand 1990; 81:253–254.
14. Smith AJ, Jackson MW, Neufing P, McEvoy RD, Gordon TP. A functional autoantibody in narcolepsy. Lancet 2004; 364:2076–2077.
15. Hinze-Selch D, Wetter TC, Zhang Y, Lu HC, Albert ED, Mullington J, Wekerle H, Holsboer F, Pollmacher T. In vivo and in vitro immune variables in patients with narcolepsy and HLA-DR2 matched controls. Neurology 1998; 50:1149–1152.
16. Matsuki K, Juji T, Honda Y. Immunological features of narcolepsy in Japan. In: Honda Y, Juji T, eds. HLA in Narcolepsy. Berlin: Springer-Verlag, 1998:150–157.
17. Strohmaier P, Mueller-Eckhardt G, Meier-Ewart K. Cellular approach for detecting narcolepsy-specific alterations in DR2 haplotypes. Hum Immunol 1998; 22:221–225.
18. Okun M, Giese S, Lin L, Einen M, Mignot E, Coussons-Read ME. Exploring the cytokine and endocrine involvement in narcolepsy. Brain Behav Immun 2004; 18:326–332.
19. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997; 82:1313–1316.
20. Black JL 3rd, Silber MH, Krahn LE et al. Analysis of hypocretin (orexin) antibodies in patients with narcolepsy. Sleep 2005; 28:427–431.
21. Black JL 3rd, Silber MH, Krahn LE et al. Studies of humoral immunity to preprohypocretin in HLA DQB1*0602 positive narcoleptics with cataplexy. Biol Psychiatry 2005; 58:504–509.
22. Black JL 3rd, Avula RK, Walker DL et al. HLA DQB1*0602 positive narcoleptic subjects with cataplexy have CSF IgG reactive to rat hypothalamic protein extract (letter). Sleep 2005; 28:1191–1192.
23. Malik S, Boeve BF, Krahn LE, Silber MH. Narcolepsy associated with other central nervous system disorders. Neurology 2001; 57:539–541.
24. Aldrich MS, Naylor MW. Narcolepsy associated with lesions of the diencephalon. Neurology 1989; 39:1505–1508.

25. Gledhill RF, Bartel PR, Yoshida Y, Nishino S, Scammell TE. Narcolepsy caused by acute disseminated encephalomyelitis. Arch Neurol 2004; 61:758–760.
26. Kubota H, Kanbayashi T, Tanabe Y, Takanashi J, Kohno Y. A case of acute disseminated encephalomyelitis presenting hypersomnia with decreased hypocretin level in cerebrospinal fluid. J Child Neurol 2002; 17:537–539.
27. Autret A, Lucas B, Henry-Lebras F, de Toffol B. Symptomatic narcolepsies. Sleep 1994; 17:S21–S24.
28. Schrader H, Gotlibsen OB, Skomedal GN. Multiple sclerosis and narcolepsy/cataplexy in a monozygotic twin. Neurology 1980; 30:105–108.
29. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
30. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, Li R. et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brain. Nature Medicine 2000; 6:991–997.

42
Metabolic Abnormalities in Human Narcolepsy

THOMAS POLLMÄCHER
Center of Mental Health, Klinikum Ingolstadt, Ingolstadt, Germany

PIERRE BEITINGER
Max Planck Institute of Psychiatry, Munich, Germany

ANDREAS SCHULD
Center of Mental Health, Klinikum Ingolstadt, Ingolstadt, Germany and Max Planck Institute of Psychiatry, Munich, Germany

I. Introduction

Although obesity has been recognized as a frequent feature in human narcolepsy already in the 1930s (1), metabolic abnormalities and their causes have rarely been studied until very recently. Early studies on an increased incidence of type II diabetes (2,28), or reduced food intake in narcoleptic subjects (3), did not trigger any major scientific interest until 1998, when the disorder was discovered to be associated with an acquired deficiency of the production of orexins (also called hypocretins). These two peptides, derived from a common precursor protein expressed almost exclusively in the lateral hypothalamus, are tightly involved in both, the regulation of sleep and wakefulness (4), and the regulation of appetite and metabolism (5). Hence, it is very likely that orexin deficiency plays a pivotal pathophysiological role in both, the sleep wake disturbances and abnormalities in endocrine and metabolic systems found in narcolepsy. The focus of the present chapter is alterations of body weight, appetite and metabolism in narcoleptic patients, whereas another chapter in this book concentrates on endocrine abnormalities (6).

II. Body Weight and Body Composition in Human Narcolepsy

The major studies reporting on weight in narcoleptic patients are summarized in Table 1. As can be seen all but one study clearly indicate that the body mass index (BMI, weight in kg divided by the height in m) is clearly and significantly elevated or, that the incidence of obesity is increased in human patients suffering from narcolepsy. Detailed studies on body composition are still missing, but one study reporting an increased waist hip ratio suggests that abdominal fat mass might be increased (7). Comparison of a small group of narcoleptic subjects to sleep apnea patients suggests a similar body composition between these two groups (Table 2).

Table 1 Studies Reporting Body Weight in Narcolepsy

Authors	Study population	Results
Daniels, 1934 (1)	Adult narcoleptic patients	Increased incidence of obesity
Bell et al., 1975 (22)	Adult narcoleptic patients	Increased carbohydrate intake
Boudoulas et al., 1983 (23)	Adult narcoleptic patients	Normal weight
Kotagal et al., 1990 (24)	Narcoleptic children	3/4 children were obese
Dahl et al., 1994 (25)	Narcoleptic children	11/16 children were obese
Schuld et al., 2000 (26)	Adult narcoleptic patients	Increased BMI
Dahmen et al., 2001 (19)	Adult narcoleptic patients And first degree relatives	Increased BMI in patients and, to a lesser extent in relatives
Schuld et al., 2002 (20)	Adult narcoleptic patients And healthy controls	Increased BMI in patients, but not HLA-DR2 positive healthy controls
Kok et al., 2003 (11)	Adult narcoleptic patients	Increased BMI, increased waist-hip ratio
Kotagel et al., 2004 (27)	Narcoleptic children	Increased BMI

It is noteworthy that three studies (Table 1) report that obesity is already present in children with narcolepsy. There are no longitudinal data available, but these studies in children and results of a yet unpublished retrospective survey in 500 patients conducted by our group suggest that weight starts to increase near to disease onset.

III. Appetite in Human Narcolepsy

Very little is known about appetite in narcoleptic patients. Reduced food intake and obesity in mice following genetic ablation of orexin neurons during postnatal development (8) suggests reduced food intake in narcoleptic patients, and, indeed one small study reports diminished food consumption compared to healthy controls (3). The aforementioned unpublished retrospective survey conducted by our group showed that in parallel to weight gain around disease onset, patients perceive increased appetite and craving for carbohydrates. These findings are not necessarily contradictory, since increased appetite when narcolepsy develops might be compensated later by reduced food intake, when obesity is established.

Table 2 BMI, Waist Circumference and Fat Mass in Narcoleptic Patients Compared to Patients with Sleep Apnea

	Narcolepsy (N = 7 male)		OSAS (N = 19 male)	
	Mean	SD	Mean	SD
BMI (kg/m^2)	29.5	3.2	29.3	3.3
Waist circumference (cm)	102.9	8.4	109.7	10.3
Fat mass (kg)	25.4	6.7	26.4	8.8

Source: From Beitinger et al., unpublished.

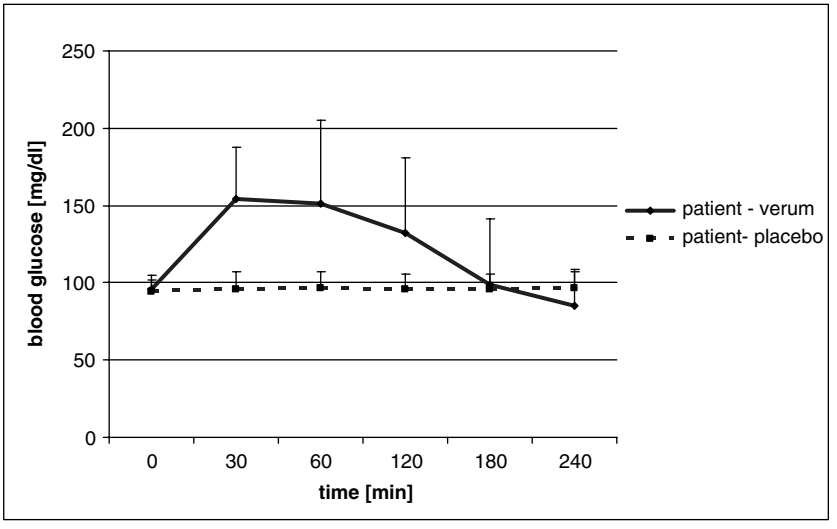


Figure 1 Blood glucose levels following the oral administration of 75 mg glucose (or a comparable placebo solution) in 15 narcoleptic patients. Eight patients displayed signs of impaired glucose tolerance defined as blood sugar level >140 mg/dl 120 min after intake.

IV. Metabolic Abnormalities in Human Narcolepsy

Although Roberts et al. (1964) and Honda et al. (1986) have reported an increased incidence of type 2 diabetes in narcolepsy (2,28), this issue has not been systematically pursued so far. An ongoing study of our group suggests that in narcoleptic patients without a history of diabetes, glucose tolerance might be impaired (Fig. 1), but at present, it is not yet clear whether this is just the consequence of increased body weight or intrinsically related to the disorder. Based on the well established fact that obesity is frequently associated with a number of other metabolic and physiologic disturbances summarized as the metabolic syndrome and associated with considerable health risks (9), it would be extremely important to know about the occurrence of these symptoms in narcolepsy. But, to date, there are no studies published investigating in this disorder blood pressure, blood uric acid, cholesterol, and triglyceride levels.

V. Causes of Alterations in Body Weight, Appetite, and Metabolism in Narcolepsy

It is tempting to speculate that orexin deficiency which probably emerges prior to, or in parallel to the occurrence of sleepiness and cataplexy causes changes in appetite, weight, and metabolic parameters in narcolepsy. However, there is very little data so far, directly supporting this assumption. Models of genetic orexin deficiency in mice (disruptions of the prepro-orexin gene or of orexin receptors) do neither induce abnormalities in weight, nor in food intake. Genetic ablation of orexin neurons taking place

during postnatal development in mice, however, was reported to be accompanied by obesity and hypophagia in adulthood (8). These findings are at least in part compatible with the data on weight and appetite in human narcolepsy, but they do not provide any insight into possible mechanisms. Endocrine studies in orexin-deficient animals have, unfortunately, not been performed so far.

In humans, two independent studies found that narcolepsy is associated with a profound deficit in the production of leptin (10,11), a hormone produced by fat cells signalling to the brain the amount of peripheral fat stores. To find decreased levels of leptin along with an increased BMI is surprising, because obesity, in general, is linked to increased leptin levels. Interestingly, leptin levels in patients with obstructive sleep apnea are increased compared to BMI matched controls (12) suggesting that hypoleptinemia in narcolepsy is not an unspecific consequence of disturbed sleep wake regulation, but rather a specific finding.

Reduced production of leptin by fat cells could be involved in the development of obesity in narcoleptic patients, because the blunted feed back to the hypothalamus might induce a dysbalance between peripheral fat mass and appetite (Fig. 2).

There is, at present, no convincing explanation why narcoleptic patients display reduced leptin levels. Adipose tissue is innervated by the autonomous nerve fibers which modulate leptin production, and orexinergic fibers impact on the autonomic nervous system as well (5). But, this impact is complex and poorly understood, so far. Those few studies which have been performed in narcolepsy on autonomic function suggest increased parasympathetic and/or decreased sympathetic activity (13). Because parasympathetic activation tends to increase leptin production (14), and sympathetic activation has been reported to do the reverse (15), these data on the activity of the ANS in narcolepsy do not explain reduced circulating leptin levels. Still, however, alterations in body weight, appetite, and metabolism in narcolepsy are most likely

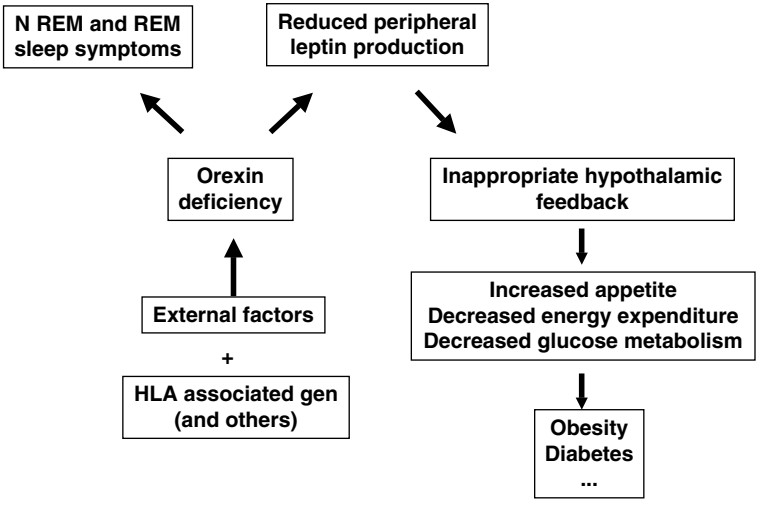


Figure 2 Possible mechanism underlying increased body weight and orexin deficiency in patients with narcolepsy (for details, see text).

caused by some pathophysiological events occurring secondary to orexin deficiency. But, some other possible causes need shortly to be discussed. First, it could be argued that sleepiness, an ensuing increased amount of sleep, and a concomitant reduction in motoric activity might per se lead to weight gain in narcolepsy due to reduced energy expenditure. However, this idea is not supported by empirical data: on the one hand narcoleptic subjects suffer from severely disturbed night sleep in addition to daytime sleepiness yielding an amount of total sleep across 24 hours not above normal (16), and on the other hand, patients display during the day increased rather than decreased amounts of motoric activity (17). Secondly, narcoleptic patients use to take medications which influence weight. Whereas stimulants tend to decrease appetite and weight, anticataplectic drugs, in particular tricyclic antidepressant, are known to lead to weight gain (18). However, although systematic prospective studies are still lacking, those studies listed in Table 1 which controlled data for the medication status suggest that weight in medicated patients is not significantly different from weight in drug free subjects. Finally, the question arises whether altered appetite, weight, and metabolism might be linked to the genetic basis of narcolepsy and, hence, not dependent of those external factors leading to orexin deficiency. This idea is supported by one study (19) suggesting that first-degree relatives of narcoleptic patients have an increased BMI. In contrast, healthy people who carry the HLA-DR2 antigen closely associated to narcolepsy have normal BMIs (20), suggesting no major genetic influence on weight in narcolepsy.

VI. Conclusions and Perspectives

Although the discovery of the pivotal role played by orexin deficiency in the pathophysiology of narcolepsy has generated growing interest in the regulation of weight and energy homeostasis, the respective knowledge still is extremely scanty. The single firmly established finding is an increased BMI, which is probably due to weight gain starting around disease onset. There is preliminary evidence suggesting that at the same time appetite increases, and carbohydrate craving occurs. But, later during the course of the disease, food intake might be even reduced. There is tentative evidence suggesting an increased incidence of diabetes type II and of impaired glucose tolerance even in patients without overt diabetes. It is unknown whether the prevalence of other symptoms of the so-called metabolic syndrome (hypertension, hyperlipidemia, hyperuricemia) is increased. However, there is urgent need to generate such data because the metabolic syndrome is associated with a strongly increased risk for cardiovascular and cerebrovascular disease, and for cancer.

Although orexin deficiency is very likely to play a pivotal role, the causes of alterations in body weight, appetite, and metabolism in narcolepsy remain unknown. Medication is unlikely to be of major importance and potential role of genetic factors has not been explored in narcolepsy. Among those endocrine abnormalities discovered so far, the reduction of leptin production by adipose tissue is one interesting candidate to contribute to an increase in appetite and weight, but at present it is neither known why leptin levels are decreased nor, whether this decrease is clinically relevant.

Unravelling the causes and peculiarities of alterations in body weight, appetite, and metabolism in narcolepsy will not only be of profound importance for the

understanding and treatment of narcolepsy, but, in addition, is likely to increase the understanding of the interaction between sleep and metabolism in general. In addition to narcolepsy, other sleep disorders such as the sleep apnea syndrome and the Kleine-Levin syndrome are associated with at least transient obesity. Moreover, numerous sedating drugs induce weight gain (18) and acute sleep deprivation was reported to impair glucose tolerance (21) further supporting the idea that disturbed sleep-wake regulation in general or hypersomnolence interact with metabolic networks in a complex manner.

References

1. Daniels LE. Narcolepsy. Medicine 1934; 13:1–122.
2. Honda Y, Doi Y, Ninomiya R, Ninomiya C. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. Sleep 1986; 9:254–259.
3. Lammers GJ, Pijl H, Iestra J, Langius JA, Buunk G, Meinders AE: Spontaneous food choice in narcolepsy. Sleep 1996; 19:75–76.
4. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. Annu Rev Psychol 2004; 55:125–148.
5. Ferguson AV, Samson WK. The orexin/hypocretin system: a critical regulator of neuroendocrine and autonomic function. Front Neuroendocrinol 2003; 24:141–150.
6. Lammers GJ, Overeem S, Pijl H. Hormonal abnormalities in hypocretin/orexin deficient human narcolepsy. In: Bassetti C, Billiard M, Mignot E, eds. Narcolepsy and other hypersomnias of central origin. New York: Marcel Dekker, 200X: X–X.
7. Kok SW, Overeem S, Visscher TL, Lammers GJ, Seidell JC, Pijl H, Meinders AE. Hypocretin deficiency in narcoleptic humans is associated with abdominal obesity. Obes Res 2003; 11:1147–1154.
8. Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K, Goto K, Yanagisawa M, Sakurai T. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001; 30:345–354.
9. Doelle GC. The clinical picture of metabolic syndrome. An update on this complex of conditions and risk factors. Postgrad Med 2004; 116:30–2, 35–38.
10. Schuld A, Blum WF, Uhr M, Haack M, Kraus T, Holsboer F, Pollmächer T. Reduced leptin levels in human narcolepsy. Neuroendocrinology 2000; 72:195–198.
11. Kok SW, Meinders AE, Overeem S, Lammers GJ, Roelfsema F, Frolich M, Pijl H. Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. J Clin Endorcrinol Metab 2002; 87:805–809.
12. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50:1511–1525.
13. Hublin C, Matikainen E, Partinen M. Autonomic nervous system function in narcolepsy. J Sleep Res 1994; 3:131–137.
14. Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, Sauerwein HP, Buijs RM. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. J Clin Invest 2002; 110:1243–1250.
15. Rayner DV, Simon E, Duncan JS, Trayhurn P. Hyperleptinaemia in mice induced by administration of the tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine. FEBS Lett 1998; 429:395–398.
16. Parkes JD, Chen SY, Clift SJ, Dahlitz MJ, Dunn G. The clinical diagnosis of the narcoleptic syndrome. J Sleep Res 1998; 7:41–52.
17. Volk S, Simon O, Schulz H, Hansert E, Wilde-Frenz J. The structure of wakefulness and its relationship to daytime sleep in narcoleptic patients. Electroencephalogr Clin Neurophysiol 1984; 57:119–128.
18. Zimmermann U, Kraus T, Himmerich H, Schuld A, Pollmächer T. Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. J Psychiatr Res 2003; 37:193–220.
19. Dahmen N, Bierbrauer J, Kasten M. Increased prevalence of obesity in narcoleptic patients and relatives. Eur Arch Psychiatry Clin Neurosci 2001; 251:85–89.

20. Schuld A, Beitinger PA, Dalal M, Geller F, Wetter TC, Albert ED, Hebebrand J, Pollmächer T. Increased body mass index (BMI) in male narcoleptic patient, but not in HLA-DR2-positive healthy male volunteers. Sleep Med 2002; 3:335–339.
21. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet 1999; 354:1435–1439.
22. Bell JI. Diet histories in narcolepsy. In:Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy – Proceedings of the first international symposium on narcolepsy 1975. New York: SP Books Division of Spectrum Publications, 1976:221–227.
23. Boudoulas H, Schmidt HS, Clark RW, Geleris P, Schaal SF, Lewis R. Anthropometric characteristics, cardiac abnormalities and adrenergic activity in patients with primary disorders of sleep. J Med 1983; 14:223–238.
24. Kotagal S, Hartse KM, Walsh JK. Characteristics of narcolepsy in preteenaged children. Pediatrics 1990; 85:205–209.
25. Dahl RE, Holttum J, Trubnick L. A clinical picture of child and adolescent narcolepsy. J Am Acad Child Adolesc Psychiatry 1994; 33:834–841.
26. Schuld A, Hebebrand J, Geller F, Pollmächer T. Increased body-mass index in patients with narcolepsy. Lancet 2000; 355:1274–1275.
27. Kotagal S, Krahn LE, Slocumb N. A putative link between childhood narcolepsy and obesity. Sleep Med 2004; 5:147–150.
28. Roberts HJ, The syndrome of narcolepsy and diabetogenic hyperinsulinism in the American negro: its relationship to the pathogenesis of diabetes mellitus, obesity, dysrhythmias, and accelerated cardiovascular disease. J Natl Med Assoc 1964; 56:18–42.

43
Hormonal Abnormalities in Hypocretin/Orexin Deficient Human Narcolepsy

GERT JAN LAMMERS
Department of Neurology and Clinical Neurophysiology, Leiden University Medical Center, Leiden, The Netherlands

SEBASTIAAN OVEREEM
Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen, and Department of Neurology and Clinical Neurophyscology, Leiden University Medical Center, Leiden, The Netherlands

HANNO PIJL
Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands

I. Introduction

The discovery that hypocretin deficiency causes narcolepsy revived the interest in possible neuroendocrine disturbances in human narcolepsy. The hypocretin system has dense projections to nuclei involved in the control of neuroendocrine networks, and a functional interaction is suggested by the association of human narcolepsy with obesity and hypoleptinemia.

In this chapter we describe our current knowledge on hormonal dysregulation in narcolepsy. First, we briefly discuss some historical data. Thereafter, we present and discuss the most relevant results of the first neuroendocrine studies that were performed in a well-defined group of hypocretin deficient narcoleptic humans and matched controls. We conclude with a hypothetical model that may explain these findings, and takes account of possible clinical consequences of the found endocrine disturbances.

II. Historical Data

Already in 1924, Redlich hypothesized that pituitary function is disturbed in patients with narcolepsy (1). Daniels described an association with obesity several years later (2). However, these early observations were made in a time when narcolepsy was not clearly defined. Sleep apnea, for example, was not recognized as a separate disease entity. Moreover, determination of the plasma concentration of many hormones was impossible.

The first (neuro) endocrine studies in narcoleptic humans were carried out in the second part of the 20th century, focusing on circulating levels of prolactin, GH, and cortisol (3–5). Unfortunately, these studies were all hampered by methodological shortcomings and/or immaturity of techniques. In the course of time, analytical techniques have greatly improved and only recently mathematical methods have been developed that allow quantitative appraisal of hormone secretion rates and mapping

of pulsatile hormone release patterns (6). Recent studies in the animal models for narcolepsy and in human patients are still scarce. The first studies in humans focused on leptin levels and changes in body weight. An increased body mass index (BMI) (7–9) and lowered serum leptin concentrations (10,11) were consistently found, although these measurements were not performed in patients with a proven hypocretin deficiency.

III. Neuroendocrine Studies in Hypocretin Deficient Narcolepsy

Seven male narcoleptic patients with typical cataplectic attacks, typical MSLT findings, and undetectable hypocretin levels in the CSF were studied. They were compared to seven controls, who were carefully matched for age, sex, BMI, and body composition. The participants were medication free. On average the subjects were overweight (BMI 28.3 kg/m^2) and the percentage total body fat ranged from 12.9% to 30.4%. Plasma concentrations of leptin, growth hormone (GH), thyroid stimulating hormone (TSH), T3/T4, adrenocorticotrope hormone (ACTH), and cortisol were measured in blood samples that were collected every 10 minutes for 24-hours. Circadian plasma profiles and hormone secretion rates were quantified using sensitive assays, pulse detection algorithms, deconvolution techniques, and cosinor fitting. Subjects remained sedentary during the studies and standardized meals were served.

The presumed physiological interactions of the hypocretin neurons with the autonomic nervous system, leptin, the adrenal, thyroid, and somatotropic as well as the SCN are represented in Figure 1.

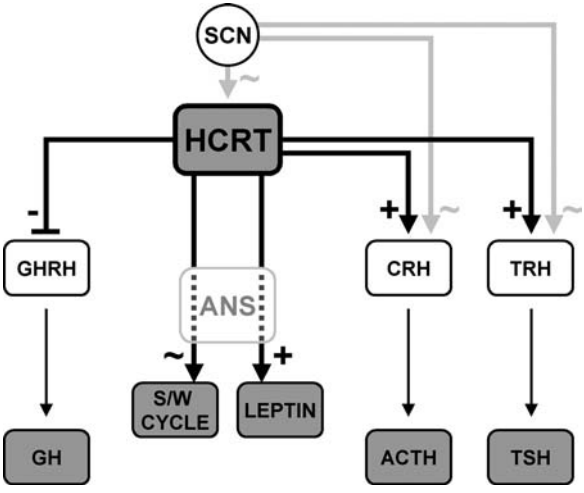


Figure 1 A model for the presumed interactions of the hypocretin neurons with the SCN, the autonomic nervous system, and the various endocrine networks. *Abbreviations:* SCN: suprachiasmatic nucleus; HCRT: hypocretin; GHRH: growth hormone releasing hormone; CRH: corticotropin releasing hormonone; TRH: thyreotropin releasing hormone; ANS: autonomic nervous system; GH: growth hormone; S/W cycle: sleep-wake cycle; ACTH: adrenocorticotropic hormone; TSH: thyroid stimulating hormone. *Symbols*: ∼, regulating/stabilizing influence; +, stimulating influence; −, inhibitory influence.

IV. Results

The most robust abnormalities were found in the secretion patterns of leptin. Leptin levels in narcoleptic patients were about half of those in normal controls during the full 24 hour period, and the normal nocturnal rise in leptin concentrations was absent in narcoleptic subjects (11). The adrenal and thyroid axes showed an almost similar pattern of disturbance: a lowered secretion on the pituitary level with a preserved rhythm and normal levels of the end-hormones (12,13). Total secretion of ACTH was lower with a preserved diurnal rhythm, whereas both secretion and rhythm of cortisol showed no difference between patients and controls (12). TSH serum levels were 50% lower in patients with a preserved diurnal rhythm, whereas the levels of T_3 and T_4 showed no difference between patients and controls (13). For the somatotropic axis, we found a increased daytime secretion of GH (14). The 24-hour mean serum concentration of GH was not significantly different in narcoleptic patients and controls, but patients secreted almost half of the total amount during the day, whereas controls produced only a quarter during daytime.

Figure 2 incorporates these results in the model of the presumed physiological interactions of hypocretin neurons (Fig. 1). It also considers possible consequences of the detected endocrine disturbances for the clinical symptomatology of narcolepsy (see also below).

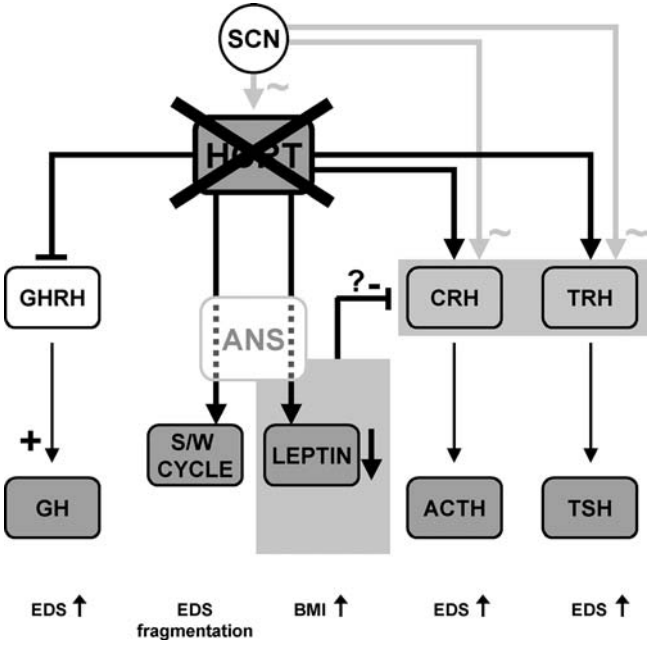


Figure 2 A hypothetical model for the endocrine disturbances in narcolepsy, and the possible clinical consequences. *Abbreviations*: As in Figure 1, EDS, excessive daytime sleepiness; BMI, body mass index.

V. Discussion

Our recent neuroendocrine studies revealed much more hormonal disturbances than previously described. The absolute levels and/or diurnal rhythm of all hormone systems studied showed differences between patients and the control subjects except in the case of cortisol, T3, and T4. The earlier found hypoleptinemia was confirmed.

Our results suggest that the suprachiasmatic nucleus (SCN) is intact in narcolepsy. This is mainly supported by the intact circadian timing of the output of the adrenal axis. However, the distribution of sleep and wakefulness, which is primarily determined by clock timing, is severely disturbed in narcoleptics. To reconcile these facts, it has been proposed that inputs from the SCN into hypocretin neurons drive clock-dependent alertness in healthy humans, and that destruction of these neurons therefore abrogates the impact of an intact master pacemaker on the circadian sleep-wakefulness cycle in narcoleptics (15). This assumption is supported by the recent finding that the diurnal hypocretin rhythm disappears in SCN lesioned animals (16,17).

The data suggest that hypocretin neurons also link SCN activity with adipocyte leptin secretion: probably via the autonomic nervous system. The impact of hypocretin neurons on the autonomic nervous system may be either direct or indirect through modulation of sleep and wakefulness.

Reduced circulating leptin levels may be involved in the pathogenesis of obesity in narcoleptic patients. The fact that narcoleptic patients are obese in the face of hypophagia suggests that they spend less energy, which is supported by early observations (2). Leptin is critically involved in the control of energy expenditure and hypoleptinemia is associated with a lower metabolic rate in obese animal models. Alternatively, hypocretin deficiency may reduce basal metabolism directly via its inhibitory impact on the sympathetic nervous system.

The adrenal and the thyroid axis showed preserved rhythms but a lowered release on the pituitary level in the face of normal concentrations of end organ hormones. The preserved diurnal rhythms of these hormones preclude that their rhythms are mediated by hypocretin neurons. However, the lowered release of ACTH and TSH may be caused by the hypocretin deficiency, as there are dense anatomical projections from the hypocretin neurons to the paraventricular nucleus, the area where CRH and TRH are synthesized (18,19). Alternatively, the lowered TSH release may be caused by the earlier discussed hypoleptinemia, which may be mediated by an inhibited TRH release (20). In addition, one might speculate whether the presumed blunted CRH and TRH release contribute to the EDS and tiredness that many patients experience (Fig. 2).

The impact of hypocretin deficiency on the somatotropic axis seems to be different. The release of GHRH is normally confined to (nocturnal) sleep. In narcoleptic patients the secretion is distributed more randomly during the 24-hour period, as is sleep. This may indicate that the high activity of hypocretin neurons during wakefulness inhibits the release of GHRH in the physiological situation (21). In the case of hypocretin deficient narcolepsy, the failing daytime inhibition leads to increased daytime GHRH and daytime sleep related GH secretion. Since GHRH is also known to induce sleepiness it is tempting to speculate whether the daytime release of GHRH in narcoleptic patients contributes to the EDS (Fig. 2).

In conclusion, the direct impact of hypocretin deficiency on the sleep-wake cycle is profound, however, the impact on endocrine rhythms, and presumably on

the autonomic nervous system may additionally affect the symptomatology of narcolepsy.

References

1. Redlich E. Über Narkolepsie. Zeitschrift Ges Neurol 1924; 95:256–270.
2. Daniels LE. Narcolepsy. Medicine 1934; 13:1–122.
3. Clark RW, Schmidt HS, Malarkey WB. Disordered growth hormone and prolactin secretion in primary disorders of sleep. Neurology 1979; 29:855–861.
4. Higuchi T, Takahashi Y, Takahashi K, Niimi Y, Miyasita A. Twenty-four-hour secretory patterns of growth hormone, prolactin, and cortisol in narcolepsy. J Clin Endocrinol Metab 1979; 49:197–204.
5. Besset A, Bonardet A, Billiard M, Descomps B, de Paulet AC, Passouant P. Circadian patterns of growth hormone and cortisol secretions in narcoleptic patients. Chronobiologia 1979; 6:19–31.
6. Veldhuis JD, Johnson ML. Analytical methods for evaluating episodic secretory activity within neuroendocrine axes. Neurosci Biobehav Rev 1994; 18:605–612.
7. Schuld A, Hebebrand J, Geller F, Pollmacher T. Increased body-mass index in patients with narcolepsy. Lancet 2000; 355:1274–1275.
8. Dahmen N, Bierbrauer J, Kasten M. Increased prevalence of obesity in narcoleptic patients and relatives. Eur Arch Psychiatry Clin Neurosci 2001; 251:85–89.
9. Kok SW, Overeem S, Visscher TL, Lammers GJ, Seidell JC, Pijl H, Meinders AE. Hypocretin deficiency in narcoleptic humans is associated with abdominal obesity. Obes Res 2003; 11:1147–1154.
10. Schuld A, Blum WF, Uhr M, Haack M, Kraus T, Holsboer F, Pollmacher T. Reduced leptin levels in human narcolepsy. Neuroendocrinology 2000; 72:195–198.
11. Kok SW, Meinders AE, Overeem S, Lammers GJ, Roelfsema F, Frolich M, Pijl H. Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. J Clin Endocrinol Metab 2002; 87:805–809.
12. Kok SW, Roelfsema F, Overeem S, Lammers GJ, Strijers RL, Frolich M, Meinders AE, Pijl H. Dynamics of the pituitary-adrenal ensemble in hypocretin deficient narcoleptic humans: blunted basal ACTH release and evidence for normal time-keeping by the master pacemaker. J Clin Endocrinol Metab 2002; in press.
13. Lammers GJ, Kok SW, Roelfsema F, Overeem S, Frolich M, Meinders AE, Pijl H. The thyreotropic axis in hypocretin deficient human narcolepsy: decreased total and pulsatile secretion of TSH (abstract). Sleep 2003; 26:A284-A284.
14. Overeem S, Kok SW, Lammers GJ, Vein AA, Frolich M, Meinders AE, Roelfsema F, Pijl H. Somatotropic axis in hypocretin-deficient narcoleptic humans: altered circadian distribution of GH-secretory events. Am J Physiol Endocrinol Metab 2003; 284:E641–E647.
15. Hungs M, Mignot E. Hypocretin/orexin, sleep and narcolepsy. Bioessays 2001; 23:397–408.
16. Deboer T, Overeem S, Visser NA, Duindam H, Frolich M, Lammers GJ, Meijer JH. Convergence of circadian and sleep regulatory mechanisms on hypocretin-1. Neuroscience 2004; 129(3):727–732.
17. Zhang S, Zeitzer JM, Yoshida Y, Wisor JP, Nishino S, Edgar DM, Mignot E. Lesions of the suprachiasmatic nucleus eliminate the daily rhythm of hypocretin-1 release. Sleep 2004; 27:619–627.
18. Peyron C, Tighe DK, Den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998; 18:9996–10015.
19. Lu XY, Bagnol D, Burke S, Akil H, Watson SJ. Differential distribution and regulation of OX1 and OX2 orexin/hypocretin receptor messenger RNA in the brain upon fasting. Horm Behav 2000; 37:335–344.
20. Nillni EA, Vaslet C, Harris M, Hollenberg A, Bjorbak C, Flier JS. Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. J Biol Chem 2000; 275:36124–36133.
21. Lopez M, Seoane LM, Tovar S, Nogueiras R, Dieguez C, Senaris R. Orexin-A regulates growth hormone-releasing hormone mRNA content in a nucleus-specific manner and somatostatin mRNA content in a growth hormone-dependent fashion in the rat hypothalamus. Eur J Neurosci 2004; 19:2080–2088.

44
Psychosocial Impact of Narcolepsy

ADRIAN M. SIEGEL
Department of Neurology, University Hospital, Zurich, Switzerland

ROGER J. BROUGHTON
Ottawa Health Research Institute, Ottawa Hospital and University of Ottawa, Ottawa, Canada

I. Narcolepsy

Narcolepsy is a common sleep disorder characterized by excessive daytime sleepiness with sleep attacks, cataplexy, hypnagogic hallucinations, sleep paralysis, and automatic behaviors. The major part of the literature on narcolepsy is focused on its etiology, pathophysiology, and treatment. Since narcolepsy has also a very significant deleterious impact on many areas of daily life the psychosocial aspects of this illness have been thoroughly studied in many studies (1–3). Comparison of the life effects of narcolepsy-cataplexy to those of matched patients with epilepsy lacking evidence for CNS lesions found the impact of narcolepsy to be as great or greater in all life areas other than education (4). Bruck compared the psychosocial impact of narcolepsy to that of three other chronic medical conditions—cardiac disease, a mixed group of cancers, and diabetes and found that the impact of was greatest for narcolepsy (5). It is also important to compare parameters to matched data from the general population. Nevsimalova and colleagues in a Czech study (6), reported that, although 81% of patients with narcolepsy were overweight, their average BMI was not different from matched population statistics; moreover, although many of their narcolepsy patients blamed educational problems on the disease, they in fact had achieved a higher average education level than the national average.

As the psychosocial impact crosses many life parameters including education, work, recreation, driving, self-esteem, interpersonal relationships, and sexuality, we will highlight the various psychosocial aspects of this sleep disorder.

II. Learning Deficits (At School, College, University)

About 6% of narcoleptics present symptoms before the age of 10 (7). The small number of narcoleptics may be the cause for the paucity of literature on psychosocial aspects in children. However, more than one-half of students with narcolepsy report learning and memory deficits that are likely secondary to diminished attentional capability as a consequence of sleepiness (8–16). Table 1 shows the various problems at school in pediatric narcoleptics.

Table 1 Psychosocial Problems at School

Falling asleep in class	52%
Achieving less than capable	44%
Poor performance	33% to 51%
Interpersonal conflicts with teachers	34%
Embarrassment due to symptoms	32%
Unable to use qualifications	30%
Frequent days off	11%
Difficulty making friends	11%

Source: From Refs. 8,16.

Besides the symptoms listed in Table 1, students with narcolepsy often suffer from irritability, fear for the future, and low frustration tolerance (17). Other symptoms include abrupt mood changes, emotional outbursts, depression, as well as other psychological problems (17). These difficulties often cause poor self-esteem and social isolation, which in turn is worsened by the lack of understanding of their teachers who frequently misinterpret the student's symptoms as laziness or malingering (8,18). In spite of these considerable problems children eventually achieve an equivalent average educational level to those without narcolepsy [6,8].

III. Difficulties at Work

Similar to preteenagers at school, adults with narcolepsy may suffer from numerous psychosocial effects of their illness at work (6,8). In questionnaire studies, sleep-related problems in the work environment have been reported by 92% of patients who suffer from narcolepsy with or without cataplexy (11). These various work problems include falling asleep on the job, fear of job loss, lower salary, and lack of promotion (Table 2). Similar to children, learning and memory deficits may be found in up to 50% of adult narcoleptics [8]. Since in formal neuropsychological memory testing narcolepsy patients may achieve similar performances as normal controls, the

Table 2 Psychosocial Problems at Work

Falling asleep at work	67% to 95%
Lost or left a job	18% to 52%
Reduced earning capacity	47%
Inability to concentrate	43%
Prevented promotion	39%
Low productivity	37%
Misunderstood by coworkers	34%
Memory problems	31% to 50%
Interpersonal problems	24%
Personality changes	18%
Accidents at work	15%
Forced disability leave	11%

Source: From Refs. 8,11,16,20.

existence of memory deficits has been questioned. However, in demanding and enduring work, narcoleptics usually show memory disturbances due to daytime sleepiness and sleep attacks (8). In a recent study, Bruck showed that younger workers with narcolepsy may have more occupational problems than older ones (5). This finding has raised the question of whether this is related to decreased symptom severity, or better accommodation of the vocational environment in older patients (5). Although in Bruck's study symptom severity did not decrease across the age, there is some indication in the literature that this effect may be the underlying cause (19). A significant portion (34%) of adults with narcolepsy reported that coworkers did not understand their health condition and viewed them as lazy, drunk or even malingering (20). Another difficulty some narcoleptics often complain about at work is their inability to know whether they will ever be able to complete an action they are initiating.

IV. Difficulties at Home and with Leisure

Activities at home may also be affected by the illness. Teixeira et al. found difficulties in cooking in 33% of patients with narcolepsy as well as problems in supervising children and in ironing in 37% and 11%, respectively (16). In addition, accidents due to narcolepsy may occur more commonly at home (in 33% of cases) compared to at work (in 15%) (16). Injuries due to accidents at home include being burnt while cooking or ironing, falls from chairs (e.g. while hanging up curtains), or even drowning in the bathtub. Smoking narcolepsy patients risk falling asleep and causing a fire with catastrophic consequences.

It is also important to ask about recreational and leisure-time activities because persons with narcolepsy report significantly more problems in both compared to controls (8). Thus, 81% of patients with narcolepsy complained about difficulties in going to the cinema, 59% in meeting friends, 48% with playing sports, and 44% in taking holidays (16). Moreover, it is obviously not advisable for patients to engage in risky hobbies such as skydiving, diving, and driving.

V. Road and Work Accidents

Another significant problem for adult narcoleptics is an increase in work-related accidents which occurred in 15% in a series of 49 patients (16). However, the incidences reported in the literature include several biases: first, they involve treated patients and, second, there is a lack of data on the types of accidents. Accidents may be minor such as wounds due to cataplectic falls. Severe accidents have also been reported, but systematic data are missing.

Beyond any doubt driving problems with an increase of accidents represent the most limiting social handicap of narcolepsy (21–22). It has been reported that 66% of patients with narcolepsy have a history of falling asleep while driving (versus 6.2% in controls), 29% have had cataplectic events (versus 0%), 67% have had near accidents (versus 0%), and 37% have had major motor vehicle accidents (versus 5.3%) (8).

VI. Problems in Relationships and Sexual Activities

Persons with narcolepsy also often suffer from difficulties in relationships. Kales et al. reported marital and family problems in 72% of patients with narcolepsy, while tension in the family occurred in 42% (23). Others have reported that, in a fifth of patients, narcolepsy causes separation or divorce (23). In a recent study of 49 narcoleptics, 56% reported relationship problems, reduced opportunities to meet partners occurred in 19%, and cessation of relations attributed to the disease was present in 30% (16). Financial problems due to reduced productivity, job loss or accidents may increase the pressure on a relationship (24).

Sexual dysfunction is often another component of the problems in interpersonal relations of persons with narcolepsy. In a study by Kales et al. 26% of narcolepsy patients reported sexual dysfunctions (23). In this study more females (36%) than males (14%) reported sexual difficulties (23). This finding, however, was questioned by subsequent studies that found either no gender difference or significantly more sexual problems in males than in females (5,8). Sexual dysfunctions include erectile problems, sleepiness, or cataplexy during intercourse, and loss of libido (25–27). Both reduction of libido in both sexes and erectile dysfunctions in males have mainly been attributed to side effects of antidepressant medications (1,5,28–29). These symptoms are common. Decreased sexual drive and/or erectile dysfunction were found in 67% of patients treated with imipramine or desipramine and in 38% if treated with imipramine or clomipramine (28,30). The negative impact of sexual dysfunctions on relationships is also shown by the fact that males with narcolepsy occasionally discontinue their antidepressant medication for this reason (26).

VII. Improving the Psychosocial Impact of Narcolepsy

Narcolepsy therefore has a profound negative impact on a patient's daily life. Many of these impairments, however, could be prevented by early diagnosis and adequate treatment of this sleep disorder. Another important aspect of medical care of patients with narcolepsy is individual counselling, in which the different potential psychosocial problems and their solutions are discussed. The latter often involve behavioural interventions which are discussed in a separate chapter.

References

1. Broughton R, Ghanem Q. The impact of compound narcolepsy on the life of the patient. In: Guilleminault C, Dement WC, Passouant P (eds) Narcolepsy. New York: Spectrum, 1976:90–93.
2. Broughton R, Nevsimalova S, Roth B. The socio-economic effects of idiopathic hypersomnia — comparison with controls and with compound narcoleptics. In: Popoviciu L, Asgian B, Badiu G, eds. Sleep 1978. Basel: Karger, 1980:229–233.
3. Goswami M, Pollak CP, Cohen FL, Thorpy MJ, Kavey MB, eds. Psychosocial aspects of narcolepsy. New York: Haworth Press, 1992 (special 1992, volume 5, edition of Loss, Grief and Care).
4. Broughton R, Guberman A, Roberts J. Comparison of psychosocial effects of epilepsy and of narcolepsy-cataplexy. Epilepsia 1984; 25:423–433.
5. Bruck D. The impact of narcolepsy on psychological health and role behaviours: negative effects and comparisons with other illness groups. Sleep Med 2001; 2:437–446.

6. Nevsimalova S, Sonka K, Spackova N, Pretl M, Homannova R. Narcolepsy: excessive daytime somnolence and psychosocial consequences. Sbor Lek 2002; 103:51–57 (in Czech).
7. Passouant P, Billiard M. The evolution of narcolepsy with age. In: Guilleminault C, Dement WC, Passouant P (eds) Narcolepsy. New York: Spectrum, 1976:179–196.
8. Broughton R, Ghanem Q, Hishikawa Y, Sugita Y, Nevsimalova S, Roth B. Life effects of narcolepsy in 180 patients from North America, Asia and Europe compared to matched controls. Can J Neurol Sci 1981; 8:299–304.
9. Levander S, Sachs C. Vigilance performance and automatic function in narcolepsy: effects of central stimulants. Psychophysiology 1985; 22:24–31.
10. Aguirre M, Broughton R, Stuss D. Does memory impairment exist in narcolepsy-cataplexy? J Clin Exp Neuropsychol 1985; 7:14–24.
11. Godbout R, Montplaisir J. All day performance measures in normal and narcoleptic subjects. Sleep 1986; 9:200–204.
12. Ollo C, Squires N, Pass H, Walsleben J, Baker T, Gujavarty K. Electrophysiological and neuropsychological assessment of cognitive function in narcolepsy. Sleep Res 1987; 16:402.
13. Rogers AE, Rosenberg RS. Tests of memory in narcoleptics. Sleep 1990; 13:42–52.
14. Schulz H, Wilde-Frenz J. Symposium: cognitive processes and sleep disturbances: the disturbance of cognitive process in narcolepsy. J Sleep Res 1995; 4:10–14.
15. Rieger M, Mayer G, Gauggel S. Attention deficits in patients with narcolepsy. Sleep 2003; 26:36–43.
16. Teixeira VG, Faccenda JF, Douglas NJ. Functional status in patients with narcolepsy. Sleep Medicine 2004; 5:477–483.
17. Kotagel S, Hartse KM, Walsh JK. Characteristics of narcolepsy in preteenaged children. Pediatrics 1990; 85:205–209.
18. Cohen FL, Ferrans CE, Smith KM. Self-esteem in persons with narcolepsy. Loss, Grief and Care 1992; 5:45–51.
19. Alaia SL. Life effects of narcolepsy: measures of negative impact, social support, and psychological well-being. Loss Grief Care 1992; 5:1–22.
20. Douglas NJ. The psychosocial aspects of narcolepsy. Neurology 1998; 50(Suppl. 1):S27–S30.
21. Findley L, Unverzagt M, Guchu R, Fabrizio M, Buckner J, Suratt P. Vigilance and automobile accidents in patients with sleep apnea or narcolepsy. Chest 1995; 108:619–624.
22. Pakola SJ, Dinges DF, Pack AI. Review of regulations and guidelines for commercial and noncommercial drivers with sleep apnea and narcolepsy. Sleep 1995; 18:787–796.
23. Kales A, Soldatos CR, Bixler EO, Caldwell A, Cadieux RJ, Verrechio JM, Kales JD. Narcolepsy-cataplexy. II. psychosocial consequences and associated psychopathology. Arch Neurol 1982; 39:169–171.
24. Broughton WA, Broughton RJ. Psychosocial impact of narcolepsy. Sleep 1994; 17 (Suppl. 8):S45–S49.
25. Roth B. Narcolepsy and hypersomnia. Broughton R, translator. Karger, Basel, 1980.
26. Karacan I. Erectile dysfunction in narcoleptic patients. Sleep 1986; 9:227–231.
27. Karacan I, Gokcebay N, Hirshkowitz M, et al. Sexual dysfunction in men with narcolepsy. Loss Grief Care 1992; 5:81–90.
28. Guilleminault C, Carskadon M, Dement WC. On the treatment of rapid eye movement in narcolepsy. Arch Neurol Psychiatry 1974; 30:90–93.
29. Gillin JC, Horowitz D, Wyatt RJ. Pharmacologic studies of narcolepsy involving serotonin, acetylcholine, and monoamine oxidase. In: Guilleminault C, Dement WC, Passouant P (eds) Narcolepsy. New York: Spectrum, 1976.
30. Takahashi Y. Proceedings: Treatment of narcolepsy, a REM sleep disorder, with psychotropic drugs. Electroencephalogr Clin Neurophysiol 1975; 39:543.

45
Narcolepsy and Driving

JEAN KRIEGER and CHRISTINE ERHARDT
Service d'Explorations Fonctionnelles, du Système Nerveux et de Pathologie du Sommeil Place de l'Hôpital, Strasbourg, France

I. Introduction

The two main symptoms of narcolepsy are likely to impair the driving capacity of narcoleptic patients. Sleepiness and sleep attacks may cause a loss of awareness of the potentially hazardous environment and a loss of control of the vehicle. Cataplectic episodes although they do not cause a loss of consciousness may impair the ability of steering the vehicle. Higher order functions (judgment and risk taking) may also be involved. Therefore, it is rather intuitive that narcoleptic patients are exposed to an increased risk of traffic accidents. This intuitive knowledge poses two questions: first, is the intuition correct? Second, which steps can be or have been taken to protect the patients and the community from the potential risk related to their impairment?

There is surprisingly little scientific evidence that these risks exist, possibly because the intuitive evidence is so compelling as to preclude scientific investigation.

The demonstration of an increased risk may stem from various sources: patient reports, administrative accident registers, evaluation of the patients' driving capacity, reports from experts examining drivers responsible for accidents. This chapter will examine the evidence based on these various sources, before it attempts to describe the current regulations concerning narcolepsy and driving in various parts of the world.

II. Evidence from Patient Reports

An early questionnaire study by Broughton et al. (1) investigating the life-effects of narcolepsy in 180 patients from North America, Europe, and Asia included questions concerning driving. Patients reported having symptoms while driving very frequently (falling asleep: 66% of patients, cataplectic episodes: 29%, sleep paralysis: 11%). A high proportion of patients admitted near or actual accidents (67% and 37% respectively). This study was replicated more recently using a similar questionnaire (2) with very similar results (see Table 1). Nevertheless, this second study reported less frequent cataplectic or sleep paralysis episodes at the wheel (16%), which seems more concordant with clinical experience.

Another study specifically oriented towards automobile accidents in patients with sleep disorders (3) compared the frequency of near or real accidents attributed to

Table 1 Proportion of Patient Having Narcolepsy Symptoms While Driving, and Having Experienced Near or Real Accidents Related to Narcolepsy Symptoms, Based on Questionnaire Data from Two Studies

	Broughton et al.		Leon-Munoz et al.		
	Narcoleptics	Controls	Narcoleptics	Controls	*P*
N	180	180	35	25	
Drive	87	113	25	25	
Fell asleep driving (%)	66.5	6.2	72	4	<0.001
Cataplexy driving (%)	28.7	0	16	0	<0.05
Sleep paralysis driving (%)	11.5	0			
(Frequent) near accidents (%)	66.7	0	64	20	<0.005
Accidents (%)	36.8	5.3	24	0	<0.01
Higher insurance (%)	16.1	0.9	16	0	NS
Suspended license (%)	12.6	6.2	4	0	NS

Source: From Refs. 1, 2.

sleepiness in 181 OSA patients, 25 narcoleptics, 35 patients with excessive daytime sleepiness of other cause, and two control groups of patients without EDS or normals. The proportions of narcoleptic patients reporting near or real accidents due to sleepiness was 72 and 52 %, respectively, higher than in any other patient group, and significantly different from controls.

Thus patient reports suggest that narcolepsy is associated with an increased risk of near or real accidents, related to the symptoms of the disorder, sleepiness, or cataplectic episodes.

However, these studies were flawed by the obvious limitation that they were based on self-reports by the patients, and could be biased by the patients' subjectivity. To our knowledge, there are no data on the frequency of car accidents in narcoleptic patients based on a more objective source of information, like accident registers.

III. Evaluation of Driving Performance

Another way to approach the patients' ability to drive is to use driving simulators. We are not aware of any study evaluating narcoleptic patients' driving performance using realistic driving simulators. The only available data were provided by studies on simplified driving simulators.

Findley et al. (4) investigated 10 patients with untreated narcolepsy versus 10 age- and sex-matched controls using the Steer Clear, a monotonous 30-min computer task in which the program displays an automobile moving on a 2-lane highway. The subjects have to move the automobile on the other lane by pressing the space bar on the keyboard in order to avoid the 780 obstacles (steers) which appear intermittently, at 2-sec to 2-min intervals. Narcoleptics were unable to avoid 7.7% of obstacles (as compared to 1.2% for controls, $p < 0.05$). Their performance was somewhat worse than that of a group of 62 OSA patients investigated in the same study (4.3 % of obstacles hit), in the same order of magnitude as that of the subgroup of severe OSA

patients. In addition, the driving records of the patients were obtained from the Department of Motor Vehicles of the Commonwealth of Virginia for 58 of the OSA patients and 9 of the narcoleptics who were licensed drivers in Virginia and had driving records over a 5-year period. A higher accident rate was associated with poorer performance on Steer Clear ($p < 0.01$), with accident rates of 0.05, 0.2, and 0.8 accident/driver/5 years in the normal (<1.8% obstacles hit), poor (1.8–4.5% obstacles hit) and very poor (>4.5% obstacles hit) performance patients, respectively. However, the small sample size did not make it possible to separate OSA and narcolepsy patients for this analysis. A later study by the same group (5) confirmed in 31 OSA patients, 16 narcolepsy patients, all untreated and 14 controls that patients had worse performance than controls. It also showed that their performance deteriorated over time much faster in narcoleptics than in OSA patients and controls when analyzing the data of the 30-minutes task by 4-minutes consecutive bins (ANOVA: effect for group: $p = 0.006$, effect for time-on-task: $p = 0.001$; group by time-on-task interaction: $p = 0.022$). Another study (6) using a divided attention test with a primary task involving tracking (a cursor controlled by a steering wheel) and a secondary task requiring visual peripheral search compared simulated driving performance in 16 narcolepsy and 21 sleep apnea patients. It showed that tracking errors were more frequent in patients than in controls (without differences between patient groups) again with a significant deterioration over time. However, the authors stressed that (1) there was only a poor correlation between tracking errors and MSLT ($r = -0.36$, $p < 0.05$ in the pooled patient group, $r = -0.32$, $p < 0.05$ in the narcoleptic group) and 2) that half of the patients performed as well as the controls.

This latter result suggests that the disorder does not impair driving performance equally in all patients. It poses the important question of whether it is possible to identify the patients at risk for driving accidents. This question is so far unanswered (7), but the suggestion is that the MSLT is not the relevant test.

IV. Expert Reports

Another way to evaluate the frequency of accidents related to a specific disorder is to analyze the frequency of the disorder among drivers involved in automobile accidents. However, there are no data available since the Department of Motor Vehicle registers do not include information on the drivers' health condition. This information may be difficult to obtain, not only for obvious privacy reasons, but also because the most interesting target population is the untreated patient population, which is by definition difficult to identify. Hopefully, once the patients are diagnosed, they are treated, and the risk is decreased, if not normalized.

Therefore, there have been little data published concerning the causes of sleepiness in drivers involved in sleepiness-related accidents. One short report (8) investigates the 110 drivers aged 18 to 70 years referred because of accidents involving bodily damage ($n = 53$) or faulty behavior at the wheel ($n = 42$) presumably due to sleepiness, in addition to 15 drivers referred by their company doctor because of sleepiness at the work place. After clinical interview and examination, PSG and MSLT the patients were categorized as:

- sleep debt, ($n = 42$, occasional in 37, chronic insomnia in 5)
- treatment with benzodiazepines ($n = 18$)

- sleep disorders ($n = 50$; 34 OSA, 11 narcolepsy, and 5 presumably idiopathic hypersomnia). Although this study probably has important selection bias (the selection criteria were poorly described), it nevertheless suggests that patients with sleep disorders are overrepresented among drivers involved in sleepiness-related accidents, and that sleepiness is not only the consequence of an occasional sleep deprivation as argued by some authors.

V. Treatment

There have been many studies showing that sleepiness and cataplexy can be improved with adequate treatment, some of which may improve performance (see the relevant chapters in this volume). However, the impact of such treatments on the actual accident risk has not been evaluated. Similarly, the effect of prophylactic measures, such as increasing the treatment dose in preparation for a driving task, or taking a prophylactic nap remain to be evaluated in this specific context.

VI. Regulations Concerning Driving and Narcolepsy

Taken together, the reported data have justified that specific legislative measures have been taken in some countries to reduce the risk of car accidents in sleepy drivers.

A. Europe

A recent report (9) has investigated the present legislation in various EU countries. The Annex III of the Directive of the Council 29.7.91 (JO 24.8.91 N° L237/1–24) applied since 1.7.1996 lists a number of disorders for which a driving license should be given only under specific conditions and under medical supervision. This list includes vision, audition, locomototor, cardiovascular, diabetes, neurology, mental, alcohol, drugs, renal, transplantation, but does not mention sleepiness nor sleep disorders. The last paragraph states that: "As a general rule a driving license should not be given or renewed to any candidate or license holder suffering from a disorder (not mentioned above) likely to compromise safety on the road, except if authorized medical advice." The directive is applied as such 9 of the 15 EU countries (the report antedates the admission of new countries), but 6 countries (B, E, F, NL, S, UK) have specific national regulations which specifically mention sleep disorders [most often OSA and narcolepsy, but in some cases insomnia (E) idiopathic hypersomnia (F)] as not being compatible with the acquisition or maintenance of a driving license. In most cases however, it is accepted that once the patient is efficiently treated he may obtain or keep his license. The definition of effective treatment is very different from country to country, as well as the periodicity of reevaluation. In addition, even in the countries where there is no specific regulation concerning sleep disorders, the drivers (or candidates to a driving license) are supposed to inform their driving authority as soon as they are aware of a condition liable to restrict their driving capacity. However, the way by which the driving authority gathers this information is highly variable, some countries require a medical certificate, established either by any doctor, or by certified doctors, others only require a questionnaire completed by the candidate himself, some

questionnaires including questions about sleep disorders, others not. Finally, the periodicity with which the driver's ability is reexamined is highly variable from country to country from never to every 2 years (often depending on the driver's age and license group).

B. United States of America

Some 10 years ago (10), there was no federal regulation concerning sleep disorders, and only a handful of states had issued restrictions for narcoleptic patients (California, Maryland, North Carolina, Oregon, and Texas). The present situation can be summarized as follows: Driving privileges are regulated at the state level in the U.S., except for commercial driving. U.S. Dept of Transportation, Highway Transportation Safety Board regulates commercial driving. Narcolepsy is an automatic disqualification for a commercial license.

In most U.S. states, driving of a personal vehicle is authorized by the Department of Motor Vehicles if a physician can certify that the patient is correctly controlled medically for his condition. In some states, for example California, as soon as a diagnosis of narcolepsy is made, the physician is instructed to report the diagnosis to the DMV and the license is temporarily suspended until the patient can prove that his condition is adequately treated with a physician certificate. In other states, there is either no regulation or physicians are instructed to report patients that are either non compliant with treatment or impossible to control medically. In a few states (California, Maryland, North Carolina, Oregon, Texas, and Utah), regulations are specific for narcolepsy, whereas in others, narcoleptic patients fall under the umbrella of conditions where loss of consciousness can interfere with driving (sleepiness, epilepsy). Physicians should always ask sleepy patients about driving and accidents and, if positive, should instruct patients not to drive until they are adequately controlled. To report or not to driving agencies is often a decision of each individual physician based on common sense and legal issues (E. Mignot, personal communication).

C. Australia

The association of Australian and New Zealand road transport and traffic authorities has issued a document, which is available on the Web. It states that "those with narcolepsy perform worse on simulated driving tasks and are more likely to have accidents than control subjects." It indicates, "the criteria for an unconditional license are NOT met" for narcoleptic patients, neither for private standards nor for commercial standards. It specifies that a conditional license may be granted subject to periodic review after consideration of the response to treatment, with more restrictive conditions for commercial standards (for which a normal sleep latency on MWT is required) than for private standards.

VII. Conclusions

There is some evidence that narcoleptic symptoms may impair driving ability, and increase the risk of traffic accidents; however, the evidence is not robust, based either on patients' self-reports, which may be biased by the patients' subjectivity, or on patients' performance on driving simulators, whose predictive value of the actual

risk of accidents is rather loose. In addition, these tests have shown that half of the patients have the same level of performance as controls, which suggests that all patients are not equally impaired. However, there is presently no test that makes it possible to identify those patients who are at risk. Nevertheless, in many states or countries, the traffic authorities have taken measures that restrict the accessibility to driving for narcoleptic patients. Whether these measures will impinge upon the frequency of sleepiness-related accidents remains to be evaluated.

From a practical point of view, although this attitude is not supported by robust data, it seems reasonable to advise sleepy drivers not to drive. Presently, the test that is most suitable for the objective evaluation for sleepiness, as it is not sensitive to cheating, is the maintenance of wakefulness test, for which normative data have recently been published (11). However, the question whether it is the physician's role to report sleepy drivers, and more generally unfit drivers, to the licensing authorities depends on the political choices made by any given country. Personally, I (JK) consider that it is the physicians' role to inform their patients about the potential risk related to their disorder and about the regulations that apply in their country, but not to force their patients if they are not willing to do so. I accept that this may be a source of conflict between the individual and the collective interest. This raises the question whether it is more ethical to report a patient to the driving authorities, or to allow him to expose himself and other drivers to an increased accident risk. Clearly, the ethics depend on the circumstances. What seemed unethical some time ago, is now perfectly accepted; similarly, some choices made in some countries would be ethically unacceptable in others. Laws and regulations in any country reflect societal choices. Our role, as experts, is to inform the authorities about the present knowledge, and to accept whatever decision is democratically made.

References

1. Broughton R, Ghanem Q, Hishikawa Y, Sugita Y, Nevsimalova S, Roth B. Life effects of narcolepsy in 180 patients fom North America and Europe compared to matched controls. Can J Neurol Sci 1981; 8:299–304.
2. Leon-Munoz L, de la Calzada MD, Guitart M. Prevalencia de accidentes en un grupo de pacientes afectos de sindrome de narcolpsia-cataplejia. Rev Neurol 2000; 30:596–598.
3. Aldrich MS. Automobile Accidents in Patients with Sleep Disorders. Sleep 1989; 12:487–494.
4. Findley L, Unverzagt M, Guchu R, Fabrizio M, Buckner J, Suratt P. Vigilance and automobile accidents in patients with sleep apnea or narcolepsy. Chest 1995; 108:619–624.
5. Findley LJ, Suratt PM, Dinges DF. Time-on-task decrements in "steer clear" performance of patients with sleep apnea and narcolepsy. Sleep 1999; 22:804–809.
6. George CFP, Boudreau AC, Smiley A. Comparison of simulated driving performance in narcolepsy and sleep apnea patients. Sleep 1996; 19:711–717.
7. George CFP. Driving simulators in clinical practice. Sleep Med Rev 2003; 7:311–320.
8. Arbus L, Tiberge M, Serres A, Rouge D. Somnolence et accidents de la circulation routière. Importance du diagnostic. Neurophysiol Clin/Clin Neurophysiol 1991; 21:39–44.
9. Krieger J, McNicholas WT, ERS Task Force. Public Health and Medicolegal Implications of Sleep Apnoea. European Respiratory Journal 2002; 20:1594–1609.
10. Pakola SJ, Dinges DF, Pack AI. Review of regulations and guidelines for commercial and noncommercial drivers with sleep apnea and narcolepsy. Sleep 1995; 18:787–796.
11. Banks S, Barnes M, Tarquinio N, Pierce RJ, Lack LC, and McEvoy RD. The maintenance of wakefulness test in normal healthy subjects. Sleep 2004 15; 27: 799–802.

46
Comorbidity in Narcolepsy

GEERT MAYER
Hephata Klinik, Schwalmstadt-Treysa, Germany

THOMAS PENZEL and KARL KESPER
Sleep Laboratory of the Department of Pneumology, Philipps-University, Marburg, Germany

I. Introduction

Narcolepsy is a rare disorder that is frequently misdiagnosed after manifestation of first symptoms. Onset of the core symptoms excessive daytime sleepiness and cataplexy can be delayed by years (7,8 years in Germany (1), 5,3 $\pm$ 9,2 years in the USA (2)). The mean delay between symptom onset and diagnosis in retrospective data in the German population has been 15.1 years (12,4–17,8 years, 95% confidence interval) but has shortened quite a bit in the past decade. Ancillary symptoms as hypnagogic hallucinations, automatic behaviour, fragmented nocturnal sleep and sleep paralysis may be misdiagnosed as nonpsychotic mental disorder, schizophrenia, disorder of the central nervous system such as epilepsy, exhaustion, collapse, vertigo, migraine etc. (3,4). Frequent co-morbid disorders may contribute to further diagnostic mismanagement and delay of diagnosis. Narcolepsy patients (np) seek help from physicians more frequently than non-affected controls (9.4 vs. 4.8) because they do not receive a satisfactory help for their main problems, eds, irresistible sleep episodes and cataplexy. They also consult more specialists (3.4 vs. 2.1) (4). Neurologists and internists most frequently diagnose narcolepsy followed by general practitioners and psychiatrists. Nowadays many narcoleptic patients find their diagnosis by publications in the media, TV and radio shows, and very often by the internet.

Medical care is quite expensive with indirect costs being much higher than direct costs (5). Impairment by narcoleptic symptoms often leads to unemployment or low income.

Besides knowledge about the classic symptoms of narcolepsy, knowledge about the co-morbid disorders may contribute essentially to an earlier diagnosis and thus to cost reduction.

So far interest in co-morbidity of narcolepsy has been sparse and has mainly been focussed on differential diagnosis and genetic association with other disorders such as diabetes mellitus (6), depression (7–9), parasomnias (9–11), obstructive sleep apnea (12,13), headache and migraine (15–17,20), and obesity (14,18,19). Only few studies looked at the spectrum of comorbid disorders in terms of prevalence (3,21).

II. Comorbidity and the German Narcolepsy Register

In order to improve the knowledge on different aspects of narcolepsy we projected a narcolepsy register for Germany. In a first step clinical data of patient records of the Hephata Klinik/Schwalmstadt Germany, which has been a narcolepsy center within the past 26 years was evaluated.

Records of 106 narcolepsy patients that were randomly chosen from more than 700 available records, were evaluated retrospectively. Data from the most recent hospital admission were chosen. Inclusion criteria differed from the ICSD-R (22) diagnostic criteria for narcolepsy by requiring cataplexies and excessive daytime sleepiness plus HLA typing positive for DR 2. In order to obtain additional data we evaluated a questionnaire on parasomnias in narcolepsy which was not completed by all NP. Therefore only numbers of patients for whom complete documentation was available are given in tables (n_i).

We assessed anthropometric data of the most recent admission to the hospital, date of narcolepsy diagnosis, symptoms and date of their manifestation, co-morbidity, medication, HLA typing, and psychosocial consequences.

106 medical records from 46 women (43,4 %) and 60 men (56,6 %) were chosen. Their anthropometric data at the most recent hospital admission are given in Table 1 (1).

The median onset of symptoms is 18,7 years. The distribution of age at onset of narcolepsy is given in Figure 1.

The distribution of the first manifestation of the major symptoms of narcolepsy is given in Figure 2.

The distribution of the three symptoms irresistible sleep episodes, cataplexy and excessive daytime sleepiness (eds) shows peaks in their frequency between 10–20 years and 40–50 years, and a minor peak between 50 and 65 years, each separated by a trough. In the first manifestation period eds is the prominent symptom, while cataplexy is prominent in the second and third period. Similar results were presented in a large franco-canadian cohort in 2001 (23) with two clear peaks in the onset of

Table 1 Anthropometric Data of 106 Narcolepsy Patients

	Mean	SD	Min	Max	SEM	$CIW_{95\%}$	n_i
Age/years, total	45,1	17,3	8	83	1,68	6,60	106
Men	46,5	15,4	13	78	1,99	7,81	60
Women	43,1	19,6	8	83	2,88	11,30	46
Height/cm, total	171	9,5	134	202	0,98	3,84	94
Men	177	7,4	158	202	1,02	3,99	53
Women	164	6,8	134	175	1,06	4,15	41
Weight/kg, total	84,1	16,2	37	130	1,62	6,34	100
Men	88,9	13,8	46	130	1,84	7,22	56
Women	77,9	17,0	37	115	2,56	10,05	44
BMI/kg·m^{-2}, total	28,7	5,0	18	45	0,51	2,01	93
Men	28,4	4,0	18	37	0,56	2,19	52
Women	29,1	5,9	19	45	0,93	3,64	41

SD, standard deviation; Min, minimum; Max, maximum; SEM, standard error of mean; $CIW_{95\%}$, confidence interval width; n_i, number of patients for whom data were available.

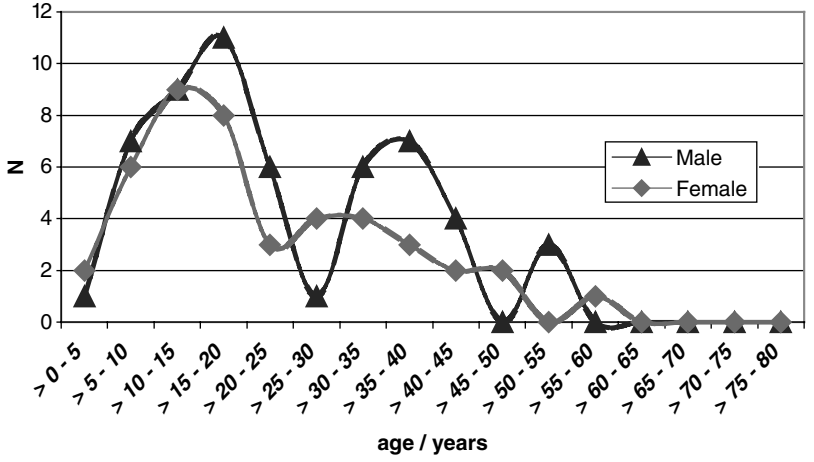


Figure 1 Distribution of age at onset of symptoms in the retro study.

symptoms, with the second smaller peak of manifestation in the late thirties. We were interested to find out if there are differences of symptom frequency between different ages of manifestation of first symptoms. According to the two most prominent peaks of age at first manifestation of core narcoleptic symptoms we divided the population in groups older and younger age 27,5 years. Patients with onset prior to 27,5 years had a significantly longer latency between first manifestation of symptoms and diagnosis than those older than 27,5 years (19,4 vs. 7,9 years, $p < 0.001$).

All diagnoses of co-morbid diseases were assessed according to ICD-10 or, if concerning sleep disorders according to ICSD-R. Depressive symptoms were confirmed if one or more symptoms of depression (i.e. depressive mood, lack of interest,

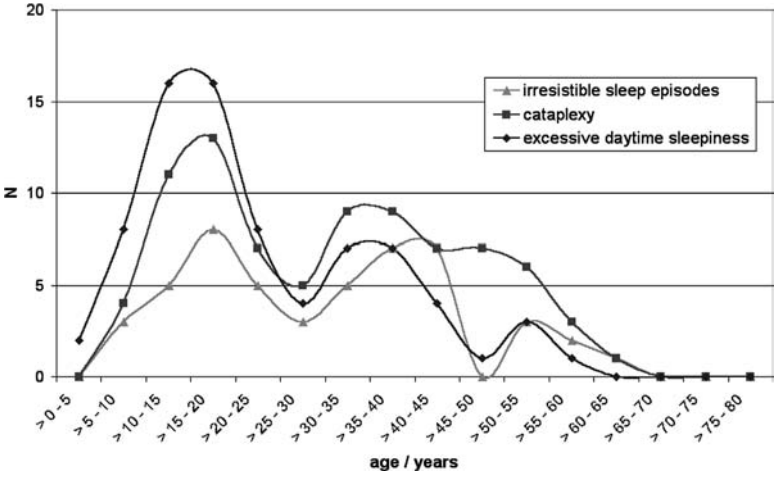


Figure 2 Distribution of manifestation of first symptoms of narcolepsy.

rumination etc.) were mentioned in the psychopathological report. Obesity was defined according to the definition of the Deutsche Gesellschaft für Ernährungswissenschaften (25) with BMI $> = 30$.

III. Comorbidity

A. Retrospective Study

In table 2 frequency of co-morbid disorders as mentioned in the medical records are listed and compared to the frequency in the general population.

Although patient records with complete listing of diagnoses are few due to unsystematic questioning some diagnoses are found very often, so that an association could be assumed. We expected them to occur more often in a prospective study in which we were using a checklist for all possible comorbid disorders. However, it is striking that sleep terrors and RBD have been diagnosed very often, which could be explained by inclusion of data from a parasomnia questionnaire for NP.

B. Prospective Study

In a prospective study we used a standardized narcolepsy questionnaire consisting of 51 questions that was sent to the patients prior to hospital admission or was handed out to them at ambulatory visits. When patients were admitted to the hospital a checklist for co-morbid diseases and actual medication was filled in by the physician. All np had polysomnographies, MSLTs and HLA typing. Np in the prospective study do not have different height and weight, but they are 7 years younger than those in the retrospective study (Table 3). The median of age at onset of symptoms is 22,5 years. Due to smaller number of patients the distribution of age at onset is 3,8 years later.

Like in the retrospective data there is a clear manifestation of first symptoms which shows three age peaks. The distribution of age at onset is the same as in the retrospective data for women, whereas it is shifted to later onset in the male np. Although the median age of onset differed in this patient group compared to retrospective data we decided to use the same split of 27.5 years as in the retrospective study. Patients with onset prior to 27,5 years had a significantly longer latency between first manifestation of symptoms and diagnosis than those older than 27,5 years (14,5 vs. 5,7 years, $p < 0.01$).

The following Table 4 shows comparison of retrospective and prospective data.

Interestingly comparison of retro- and prospective data differs in only few comorbid diseases. Sleep terrors, sleeptalking and PLMD are less frequent, internistic diseases as hypertension and diabetes are more frequent than in the retrospective study.

In both the retro- and prospective study the number of comorbid diseases shows a significant correlation with age. However, ruling out the influence of age on the correlation of the duration of narcolepsy this correlation is no longer significant.

IV. Parasomnias

In 1983 Reynolds et al. (40) reported that 52% of all narcolepsy patients had parasomnias like sleepwalking, bruxism, and enuresis. In 1993 Mayer et al. (11) found that

Table 2 Case Numbers and Minimal Frequencies of Diagnoses Compared to Results of Studies of Comorbidity and Prevalence

	Total $n_{tot} = 106$		Other comorbidity studies		Prevalence in the population	
Comorbidity	n_i	f_{min}	F	Reference	F	Reference
Parasomnias						
Disorders of arousal						
Sleep walking	15	14,2%	8%	Sturzenegger, Bassetti. 2004 [24]	2,5%	Klackenberg [26]
Sleep terrors	9	8,5%	–	–	–	
Sleep wake transition disorders						
Sleeptalking	27	25,5%	63%	Sturzenegger, Bassetti. 2004 [24]	–	
REM-parasomnias						
Nightmares	44	41,5%	54%	Sturzenegger, Bassetti. 2004 [24]	5-7%	Wood and Bootzin [27]
REM-behaviour disorder	20	18,9%	–	–	0,5%	Mahowald 2000[10]
Other parasomnias						
Bruxism	12	11,3%	–	–	6–12%	Gross et al. [28]
Enuresis	7	6,6%	–	–	1,5-3%	Tietjen and Husman [29]
Intrinsic Sleep Disorders						
SBAS / OSAS	19	17,9%	13%	Baker et al. [12]	4%	Young et al. [30]
PLMD	15	14,2%	40%	Boivin et al. [30]	5–30%	Ancoli-Israel et al. [32]
Restless Legs syndrome	1	0,9%	43%	Sturzenegger, Bassetti. 2004 [24]	5–10%	Trenkwalder et al. [33]
Internistic Diseases						
Obesity	37	34,9%	32%	Schuld et al. [18], Dahmen et al. [20]	11-12%	Heseker et al. [34]
Neurological Diseases						
Headache	15	14,2%	81%	Dahmen et al. [14]	78%	Rasmussen et al. [35]
Epilepsy	3	2,8%	–	–	1%	Keränen, Rickkinen [36]
Hyperkinetic syndrome	3	2,8%	–		16%	Rowland et al. [37]
Psychiatric Diseases						
Depressive symptoms	16	15,1%	–		2,5%	Wittchen et al. [38]
Depression	8	7,6%	18-70%	Broughton et al. [7], Douglass et al. [39]	11,5%	Wittchen et al. [38]

n_i, number of patients in whom the disorder was reported; n_{tot}, total number of the respective sample; Ref, reference. SRBD, sleep related breathing disorder; OSAS, obstructive sleep apnea syndrome; PLMD, periodic limb movement disorder.

Table 3 Anthropometric Data of 56 Narcolepsy Patients

	Mean	SD	Min	Max	SEM	$CIW_{95\%}$	n_i
Age/years, total	38,5	12,24	17	68	1,65	6,41	56
Men	41,9	7,9	31	57	1,62	6,34	24
Women	35,7	14,2	16	68	2,52	9,86	32
Height/cm, total	171,4	8,36	152	193	1,12	4,38	56
Men	176,6	7,58	164	193	1,53	6,06	24
Women	167,5	6,72	152	182	1,19	4,66	32
Weight/kg, total	84,45	20,18	53	150	2,72	10,66	55
Men	94,1	21,40	63	150	4,46	17,49	23
Women	77,53	16,31	53	106	2,88	11,29	32
BMI/kg·m^{-2}, total	28,62	5,89	20,5	44,8	0,8	3,1	55
Men	29,88	5,23	22,6	40,7	1,09	4,27	23
Women	27,7	6,26	20,51	44,8	1,11	4,33	32

The distribution of the age at onset is given in Figure 3.

Table 4 Comparison of Retrospectively and Prospectively Obtained Comorbidity in Narcolepsy Patients

	Total $n_{tot} = 106$		Total $n_{tot} = 56$		
Comorbidity	n_i	f_{min}	n_i	n	F
Parasomnias					
Disorders of arousal					
Sleep walking	15	14,2%	8	55	14,5%
Sleep terrors	9	8,5%	0	55	0%
Sleep wake transition disorders					
Sleeptalking	27	25,5%	7	55	12,7%
REM-parasomnias					
Nightmares	44	41,5%	18	55	32,7%
REM-behaviour Disorder	20	18,9%	10	55	17,8%
Other parasomnias					
Bruxism	12	11,3%	6	55	10,9%
Enuresis	7	6,6%	0	55	0%
Intrinsic Sleep Disorders					
SBAS / OSAS	19	17,9%	10	54	18,5%
PLMD	15	14,2%	5	54	9,3%
Restless Legs syndrome	1	0,9%	2	55	3,6%
Internistic Diseases					
Obesity	37	34,9%	34	55	38,2%
Hypertension	5	4,7%	9	56	16,1%
Diabetes	5	4,7%	3	56	5,4%
Asthma	3	2,8%	6	56	10,7%
Neurological Diseases					
Headache	15	14,2%	10	56	17,9%
Epilepsy	3	2,8%	0	56	0%
Hyperkinetic syndrome	3	2,8%	0	55	0%
Psychiatric Diseases					
Depressive symptoms	16	15,1%	nd		Nd
Depression	8	7,6%	11	55	20%

n_i, number of patients in whom the disorder was reported; n_{tot}, total number of the respective sample; nd, not done.

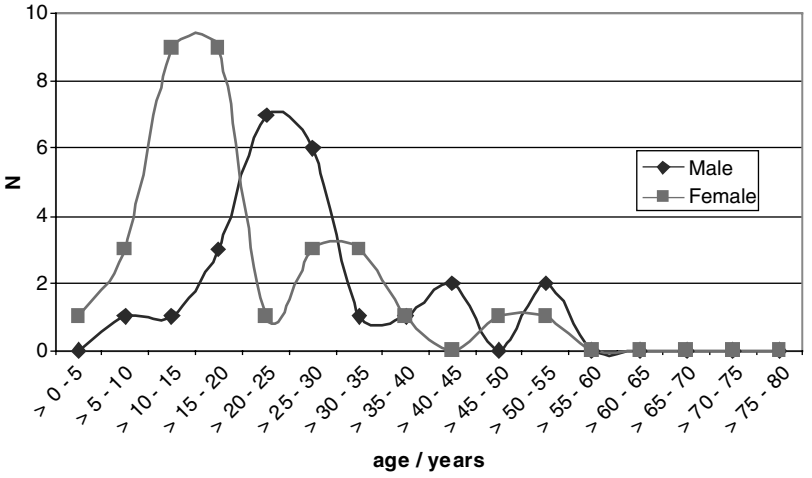


Figure 3 Distribution of age at onset of narcolepsy.

many NP had one or more parasomnias often starting during childhood and preceding narcolepsy, with some of the patients switching from one parasomnia to the other or having some of them at the same time.

The present data confirms these findings. So far only few studies were reporting parasomnia in NP (10,24,40,41). The high percentage of np suffering from REM- and NREM parasomnias suggests a common pathway. In a study of multicase families we found that unaffected and affected first and second-degree relatives of NP had a higher frequency of parasomnias compared to the frequency of narcoleptic symptoms. HLA DQB1*0602 was more frequent in first-degree relatives of NP with parasomnias than in those with EDS (41).

In a recent genetic study on sleepwalkers families with several affected members had a significant excess transmission for DQB1*05 (42).

A higher incidence of REM sleep behaviour disorder (RBD) in narcolepsy has first been reported by Mahowald and Schenck (10) and was interpreted as expected, because both disorders are characterized by "state boundary control" abnormalities. Again in this REM-parasomnia HLA DQBw1 was found frequently (43). As HLA DQB1 genes are frequent in several parasomnias and in narcolepsy they appear to be strongly involved in disorders of motor control during sleep. Since parasomnias are much more prevalent in np than in controls an association of the diseases can be assumed.

The high frequency of nightmares is not surprising as narcolepsy is a disorder of REM-sleep. Another background might be the association of nightmares with psychiatric disorders. As depression is quite prevalent in NP a high frequency of nightmares could be assumed. However, depression may be regarded a sequel of the chronic course of narcolepsy (44) and not as a primary disorder. The altered REM-sleep in depression could, however in part explain the high frequency of nightmares.

The frequency of bruxism and enuresis are about as high as in the general population (28). Bruxism is seldom reported by patients, since it is considered a dental rather

than a sleep problem. Np normally do not report bruxism, it is mainly detected by polysomnography.

Since the discovery of orexins new knowledge has been accumulated on the regulation of sleep, autonomous and motor control. A deficiency of orexins may cause imbalance of the cholinergic and noradrenergic brainstem regulation initiating an instability of REM (45) resulting in an increased REM-tone. It has been recognized that orexins are involved in the regulation of motor activity. In which ways the lack of orexins exerts an influence in causing parasomnias is not clear until today.

V. Intrinsic Sleep Disorders

An association between obstructive sleep apnea and narcolepsy has been reported, the authors refer to different types of sleep related breathing disorders (12), (13). Baker et al (12) found that 13% of their patients had OSAS with an apnea index $>5/h$ which is much higher than in the general population. Apparently comorbid OSAS does not influence daytime sleepiness (46).

In our own data frequency of SRBD was 16% which is much higher than the 4% given for a control population (30). SRBD is not only prevalent in NP beyond the median age of onset of 22,5 years (20–22%) but also in those below the median onset of age 22.5 years (12,2–14,8%). The pathogenetic background so far has never been investigated. A plausible explanation is the obesity plus frequent muscle atonia in NP, which might predispose for SRBD. The prospective data shows increased frequency of hypertension in NP (5–11% in the age group below 22,5 years, 13–21% in the age group older than 22,5). Distribution of SRBD and hypertension even at young age predisposes NP to an increase cardiovascular risk.

The frequency of periodic limb movement (PLM) with 14.25 was much lower than that reported by other authors (40% (31), (47), (48)), but much higher than in the general population. It is less frequent in the group with age at onset below 22.5 years (3.6% vs. 15.4 %). Considering the mean age of our NP we were actually expecting no more than a prevalence of 5% for the general population (33). We therefore conclude that there is a comorbidity. PLM with arousal or awakening is less frequent in NP with cataplexy than in those without (49). For NP additionally suffering from sleep apnoea it remains unclear if arousal associated with PLM is triggered by apnoea or PLM (29). PLMD is tightly associated with RLS. Both sleep disorders may occur less frequent in NP, because most of the patients have undetectable orexin levels in their cerebrospinal fluid, whereas it is normal in patients with idiopathic RLS (50). Sturzenegger und Bassetti (24) found RLS in 43% of their NP. The enormous discrepancy to our finding of 0.9% NP with RLS might be caused by different methods used. Whereas Sturzenegger and Bassetti used a questionnaire we used a check-list for co-morbid diseases in a personal interview. Possibly NP have difficulties attributing somatosensor perceptions to certain symptoms.

VI. Internistic Diseases

Obesity (BMI $>=30$) in our retrospective investigation was found in 34.9% of all np, which is much higher than in the german population (11–12%, (35)). An association

between narcolepsy and obesity has been shown in several studies (18–20), some authors even (20) suggested a narcolepsy-obesity syndrome. In one study of NP and non-affected relatives the latter have a higher BMI (18), (14) than the general population. Kok et al. (19) have found reduced plasma leptin levels with a missing nocturnal acrophase in NP. They suggest that the influence of disrupted circadian distribution of sleep and/or disrupted signalling of the SCN on sympathetic tone, caused by orexin deficiency, are responsible for the disturbance of the leptin circadian rhythm in NP. The reduced plasma levels may predispose NP to gain weight.

The high number of NP suffering from asthma, especially in the group with onset of symptoms prior to age 22.5 years (14.3% vs. 7.1%) remains unclear and has to be monitored in larger patient populations.

VII. Neurological Diseases

14.2% of our NP suffered from headache, none from migraine. This frequency is much lower than that reported in the German general population (28–86% (35) and by Dahmen et al. (14), who found headache in 81% (tension headache 21%), and migraine in 54 %. In a confirmative study of 100 NP with a computerized system 44% of the females and 28.3 % of the males had migraine (15). In a case-control study Evers found tension headache in 60.3 % NP compared to 40.7% in the controls, migraine in 21.9% NP and 19.8% controls. In contrast to Dahmen et al. (17) the authors concluded that there is no association between narcolepsy and headache and that headache could be caused by sleep disruption in NP. Both studies were discussed by Lammers, who suspected on one hand drug treatment with amphetamines to be in part responsible for the headache, on the other hand the complaint of headache prior to cataplectic attacks, which is reported by many NP. Another reason for headache could be the high rate of sleep related breathing disorders which are often accompanied by morning headaches. In patients who did not receive standardized headache questionnaires diagnoses were based on anamnesis. We might therefore have missed a correct diagnosis in some patients, who apparently do not classify their headache as being primary.

The hyperkinetic syndrome may be an important differential diagnosis in children (37), and therefore should lead to enforced investigation for narcoleptic symptoms. However, the number of NP with this diagnosis is too small to conclude any co-morbidity.

An association of epilepsy and narcolepsy is extremely rare ((51), (52)). Again our patient umbers are too small to assume an increased frequency in NP compared to the general population (prevalence of 1%).

VIII. Psychiatric Disorders

A. Depression and Depressive Symptoms

Affective disorders are frequent in narcolepsy. Broughton et al. (7) reported that 51.1 % of NP suffer from depression, Aldrich (53) found it in 30%, Douglas et al. (39) in 69.8%. Merritt (8) found depressive symptoms in 49%. Other authors reported a

much lower prevalence of 17,2% (9). The frequency given in literature has to be looked at critically. Most data is based on questionnaires and not on DSM-III or IV classification. Impaired self esteem in NP may be a confounding factor. Authors using DSM-III found depression in 22% (54) with no significant differences compared to controls (57) who additionally used the POMS compared several different sleep disorders and found depressive symptoms to be significantly higher than in patients with sleep apnoea. Depressive symptoms often start prior to onset of narcolepsy and are more severe in NP with a short history of narcolepsy than in those with a long history, which might be depending on better coping strategies (56). Our own data shows a comparable percentage of NP affected with depressive symptoms and depression compared to the authors citing DSM III classified diagnoses.

Narcolepsy and depression have been investigated for possible common features of REM sleep (55). Comparing depressive and narcoleptic patients there was no difference in total amount of REM-sleep, mean REM density, mean REM latency, but significant difference in REM latency distribution (in 54% at sleep onset, in 46% starting $>$60 min. after in narcoleptics; in depressives none at sleep onset, 65% from 1-60 min. with equal distribution, sign. age effect of REM latency). It was concluded from this study that the mechanisms of REM sleep disinhibition are different.

IX. Conclusion

Investigation of comorbid disorders in narcolepsy in our German sample led to some new results: Parasomnias, sleep apnoea and obesity are much more frequent than reported in the literature so far, whereas PLMD, restless legs syndrome, headache and depression are less frequent. Narcolepsy thus is associated with many other diseases mainly sleep disorders. Obesity seems to be another hallmark, occurring especially at the onset of narcolepsy and progressing with age. These comorbid disorders often manifest prior or at the same time as symptoms of narcolepsy evolve and sometimes share a common pathophysiology. The manifold occurrence of these comorbid diseases compared to the general population clearly points out that there is an association.

Earlier diagnosis and treatment will result in empowering greater quality of life, productivity and lowering costs. Especially in children and patients with monosymptomatic narcolepsy, and those with early onset knowledge about comorbid diseases may contribute to establish the diagnosis narcolepsy faster as in the past. Furthermore it may help to diagnose cases of mild narcolepsy and lower the number of unrecognized cases.

References

1. Mayer G.; Kesper K.; Ploch T.; et al. The implications of gender and age at onset of first symptoms in narcoleptic patients in Germany – results from retrospective evaluation of hospital records. Somnology 2002, *6* (1), 13–18
2. Silber M.H.; Krahn L.E.; Olson E.J.; et al. The epidemiology of narcolepsy in Olmsted County, Minnesota: a population-based study. Sleep 2002, *25* (2), 197–202
3. Mayer G.; Peter H.; Kesper K.; et al. Komorbidität bei Narkolepsiepatienten. Dtsch. Med. Wochenschr. 2002, *127*, 1942–46

4. Kryger M.H.; Walid R.; Manfreda J. Diagnoses received by narcolepsy patients in the year prior to diagnosis by a sleep specialist. Sleep 2002, *25*(1), 36–41
5. Dodel R.; Peter H.; Walbert T.; et al. The socioeconomic impact of narcolepsy. Sleep 2004, 6, 1123–1128
6. Honda Y.; Doi Y; Ninomiya R; et al. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. Sleep 1986, *9*, 254–259
7. Broughton R.; Ghanem Q.; Hishikawa Y.; et al. Life-effects of narcolepsy: relationships to geographic origin (North America, Asia and Europe) and other patient and illness variables. Can. J. Neurol. Sci. 1983, *10*, 100–104
8. Merritt S.L.; Cohen F.L.; Smith K.M.. Depressive symptomatology in narcolepsy. In *Psychosocial Aspects of Narcolepsy*. Goswami M, Pollak C.P., Cohen F.L, Thorpy M.J., Kavey N.B. (Ed.). The Haworth Press Inc.: New York 1992, 53–59
9. Roth B.; Nevsímalová S. Depression in narcolepsy and hypersomnia. Schweizer Archiv für Neurologie, Neurochirurgie und Psychiatrie 1975, *116* (2), 291–300
10. Mahowald M.W.; Schenck C.H. REM sleep parasomnias. In Kryger M.H.; Roth T.; Dement W.C., ED. In *Principles and Practice of Sleep Medicine*. 2nd ED.; W.B. Saunders Company: Philadelphia: 2000, 724–741
11. Mayer G, Meier-Ewert K. Motor dyscontrol in sleep of narcoleptic patients (a lifelong development?) Journal of Sleep Research 1993, *2* (3), 143–148
12. Baker T.; Guilleminault C.; Nino-Murcia G; Dement W.C. Comparative polysomnographic study of narcolepsy and idiopathic central nervous system hypersomnia. Sleep 1986, *9*,232–242
13. Chokroverty S. Sleep apnea in narcolepsy. Sleep 1986, *9*(1), 250–253
14. Dahmen N.; Querings K.; Grün B.; et al. Increased frequency of migraine in narcoleptic patients. Neurology 1999, *52*, 1291–1293
15. Dahmen N.; Kasten M.; Wieczorek S; et al. Increased frequency of migraine in narcoleptic patients: a confirmatory study. Cephalgia 2003, *23*, 14–19
16. Lammers G.J. Are headache and narcolepsy associated? Cephalgia, 2003, *23*, 775
17. The DMKG Study Group. Migraine and idiopathic narcolepsy – a case-control study. Cephalgia 2003, *23*, 786–789
18. Schuld A.; Hebebrand J.; Geller F.; et al. Increased body-mass index in patients with narcolepsy. The Lancet 2001, *355*, 1274–1275
19. Kok, S.W.; Meinders A.E.; Overeem S.; et al. Reduction of plasma leptin levels and loss of ist circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. J. Clin. Endocrinol. Metab. 2002, *87*, 805–809
20. Dahmen N.; Bierbrauer J.; Kasten M. Increase prevalence of obesity in narcoleptic patients and relatives. Eur. Arch. Psychiatry Clin. Neurosci. 2001, *251*, 85–89
21. Rosa R.; Kramer M.; Ebrigth P. Narcolepsy: symptom frequency and associated disorders. Sleep Res. 1980, *8*, 213
22. ICSD Revised - International classification of sleep disorders: Diagnostic and coding manual. Diagnostic Classification Steering Committee, Chairman MJ Thorpy. Rochester, Minnesota. American Sleep Disorders Association 1997
23. Dauvilliers Y, Montplaisir J, Molinari N, Carlander B, Ondze B, Besset A, Billiard M. Age at onset of narcolepsy in tow large populations of patients in France and Quebec. Neurology 2001;57:2029–2033
24. Sturzenegger C, Bassetti C. The clinical spectrum of narcolepsy with cataplexy: a reappraisal. J Sleep Res 2004;13:395–406
25. Hebebrand J.; Himmelmann G.; Heseker H.; et al. Percentiles for the body mass index based on data of the German National Nutrition Survey and a review of relevant factors with an influence on body weight. Aktuelle Ernährungsmedizin 1994, *15*, 247–252
26. Klackenberg G. Somnambulism in childhood: prevalence, course and behavioral correlations. Acta Paediatr. Scand. 1982, *71*, 495–499
27. Wood J.; Bootzin R.R. The prevalence of nightmares and their independence from anxiety. J Abnorm. Psychol. 1990, *99*, 64–68
28. Gross A.J.; Rivera-Morales W.C.; Gale E.N. A prevalence study of symptoms associated with TM disorders. J. Craniomandib. Disord. Facial Oral Pain 1988, *2*, 191–195
29. Tietjen D.N.; Husman D.A. Nocturnal enuresis: A guide to evaluation and treatment. Mayo Clin. Proc. 1996, *71*, 857–862

30. Young T.; Palta M.; Dempsey J.; et al. The occurence of sleep disordered breathing among middle-aged adults. N. Engl. J. Med. 1993, *328*, 1230–1235
31. Boivin D.B.; Montplaisir J.; Petit D.; et al. Effects of modafinil on symptomatic human narcolepsy. Clin. Neuropharmacol. 1993, *16*, 46–53
32. Ancoli-Israel S.; Kripke D.M.; Klauber M.R.; et al. Periodic limb movements in sleep in community dwelling elderly. Sleep 1991, *14*,496–50
33. Trenkwalder C.; Wetter T.C.; Stiasny K.; et al. Restless legs Syndrom und "periodic limb movements in sleep". Nervenarzt 2001, 6, 425–436
34. Heseker H.; Hartmann S.; Kubler W.; et al. An epidemiologic study of food consumption habits in Germany. Metabolism 1995, *44*(Suppl 2), 10–13
35. Rasmussen B.K.; Jensen R.; Olesen J. Impact of headache on sickness, absence and utilization of medical services. J. Epidemiol. Community Health 1992, *46*, 443–446
36. Keränen T.; Rickkinen P. Severe epilepsy: diagnostic and epidemiological aspects. Acta Neurol. Scand. 1988, *57*(Suppl 17), 7–14
37. Rowland A.S.; Umbach D.M., Catoe K.E.; et al. Studying the epidemiology of attention-deficit hyperactivity disorder: screening method and pilot results. Can. J. Psychiatry 2001, *46*, 931–940
38. Wittchen H.U.; Müller N.; Schmidtkunz B.; et al. Erscheinungsformen, Häufigkeit und Versorgung von Depressionen. Fortschr. Med. 2000, *118*(Sonderheft1), 4–10
39. Douglas, Merritt S.L.; Cohen F.L.; et al. Depressive symptomatology in narcolepsy. Goswami M.; Pollak C.P.; Cohen F.L.; et al., Ed. In *Psychosocial aspects of narcolepsy*. The Haworth Press Inc.: New York, 1992, 53–59
40. Reynolds C.F. III; Christiansen C.L.K Taska L.S.K et al. Sleep in narcolepsy and depression – does it all look alike? J. Nerv. Ment. Dis. 1983, *171*, 290–295
41. Mayer G.; Lattermann A.; Mueller-Eckhardt J.; et al. Segregation of HLA genes in multicase families. J. Sleep Res. 1998, *7* (2), 127–134
42. Lecendreux M.; Mayer G.; Bassetti C.; et al. HLA and genetic susceptibility to sleepwalking. Molecular Psychiatry 2003, *8*, 114–117
43. Schenck C.H.; Garcia-Rill E.; Segall M.; et al. HLA class II genes associated with REM sleep behavior disorder. Ann. Neurol. 1996, *39*, 261–263
44. Kales A, Soldatos CR, Bixler EO, Caldwell A, Cadieux RJ, Jeffrey M, Verrechio M, Kales JD. Narcolepsy-cataplexy. II. Psychosocial consequences and associated Psychopathology. Arch Neurol 1982,39, 169–171
45. Hungs M.; Mignot E. Hypocretin/orexin, sleep and narcolepsy. Bio Essays 2001, *23*, 397–408
46. Inoue Y.; Nanba K.; Higami S.; et al. Clinical significance of sleep-related breathing disorder in narcolepsy. Psychiatry Clin. Neurosci. 2002, *56*, 269–270
47. Harsh J.; Peszka J.; Hartwig G.; et al. Night-time sleep and daytime sleepiness in narcolepsy. J Sleep Res 2000, *9*, 309–316
48. Young D.; Zorick F.; Wittig R.; et al. Narcolepsy in a pediatric population. AJDC 1988, *142*, 210–213
49. Moscovitch A.; Partinen M.; Guilleminault C. The positive diagnosis of narcolepsy and narcolepsýs borderland. Neurology 1993, *43*, 55–60
50. Stiasny-Kolster K.; Mignot E.; Ling L.; et al. CSF hypocretin-1 levels in restless legs syndrome. Neurology 2003, *61*(10), 1426–1429
51. Tharp B.R. Narcolepsy and epilepsy. In *Narcolepsy*; Guilleminault C., Dement W.C.; Passouant P., Ed: Spectrum: New York, 1976, 263–281
52. Bonduelle M, Degos C. Symptomatic narcolepsy: a critical study. In: Guilleminault C, Dement WC, Passouant P (Eds). Narcolepsy. New York, Spectrum, 1976, 313–332
53. Aldrich M.S. Narcolepsy. Neurology 1992, *42* (suppl. 6), 34–43
54. Wilcox J. Psychopathology and narcolepsy. Neuropsychobiology 1985, *14*, 170–172
55. Pollmächer T.; Mullington J.; Lauer C. REM sleep disinhibition at sleep onset: a comparison between narcolepsy and depression. Biol. Psychiatry 1997, *42*, 713–720
56. Aksu S. Bewältigungsverhalten von Narkolepsiepatienten. Diplomarbeit am Fachbereich Psychologie der Philipps-Universität Marburg, 1997
57. Mosko S.; Zetin M.; Glen S.; et al. Self-reported depressive symptomatology, mood ratings, and treatment outcome in sleep disorders patients. Journal of Clinical Psychology, 1989, *45*(1), 51–60

47
The Behavioral Management of Narcolepsy

ROGER J. BROUGHTON
Ottawa Health Research Institute, Ottawa Hospital and University of Ottawa, Ottawa, Canada

BRIAN J. MURRAY
Sunnybrook and Women's College Hospital, University of Toronto, Toronto, Canada

I. Introduction

Behavioral management may be defined as those actions by a medical practitioner, his patient or others that do not directly involve the taking of medication or the use of medical therapeutic devices and that help treat, alleviate or ameliorate, the consequences of a disease or dysfunction.

The behavioral management of narcolepsy is important for a number of reasons. First, although the REM sleep-based symptoms can usually be well controlled by medication, this is certainly not the case for excessive daytime sleepiness (EDS) which, once established, is almost never totally reversed (1,2). Secondly, EDS is the principal symptom to which the many psychosocial consequences of the disease are attributed by patients (3). This attribution appears correct as an almost identical life effects profile has been reported for idiopathic hypersomnia which has similar levels of sleepiness but lacks the REM-based symptoms (4) and because modafinil, whose effect is on wake maintaining systems and not on sleep, reduces these effects (5). Third, behavioral management initiatives lead to a sense of some degree of mastery or at least of partial control of the disease and its impact, and indeed they can significantly help the patient in coping with the disease. Finally, there have been a few patients for whom behavioral interventions alone at the very onset of the disease have led to apparent reversal of the disease. One of us (RB) was consulted on an adolescent female student who presented with EDS, involuntary sleep attacks, typical cataplexy and SOREMPs and who, like many of her age, was following very irregular night bed hours. After regular night sleep had been established all symptoms of narcolepsy disappeared; and these were still absent 10 years later, when the patient was last seen.

Despite the important role of behavioral initiatives in the management of narcolepsy there are to our knowledge only two published reviews of the topic (6,7) supplemented by two reports documenting the strategies that patients state they use (8,9). Although for most behavioral interventions there are no controlled studies of efficacy, it would appear reasonable to employ any for which there is a rationale that makes sense in our current state of knowledge or for which there exists strong anecdotal evidence of efficacy. Although physicians often do not think to recommend behavioral

interventions, their value is certainly recognized by patients, only 10-15% of whom rely exclusively on stimulant medication for control of daytime sleepiness (10).

The available approaches will be considered according to whether they focus upon the main nocturnal or diurnal symptoms, on symptoms secondary to EDS, on psychiatric aspects, obesity, psychosocial impact areas, co-existent sleep disorders, counseling or educational needs. A brief review of the relevant literature for each aspect precedes consideration of available behavioral management approaches other than for the psychosocial impact which is reviewed elsewhere in this volume.

II. Night-Time Symptoms

A. Nocturnal Dysomnia

Sleep maintenance insomnia is common and can be severe. Insomnia typically begins within one to five years of the appearance of EDS, often around the time of onset of the REM-based symptoms of cataplexy, sleep paralysis, and vivid hypnagogic hallucinations (11,12). There is no overall close correlation between the amount of night sleep or night sleep efficiency and subsequent daytime sleep latencies (13) or amount of day sleep (14) in narcolepsy. Never-the-less, as for normal persons, one or several nights of significant sleep deprivation will increase daytime sleepiness levels (15). Yoss and Daly (16) stated that improving night sleep quality is particularly efficacious in younger patients. As cataplexy is facilitated by increased daytime drowsiness and responds somewhat to stimulant medication (17), one might reasonably expect some improvement in this pathognomonic symptom as well. Indeed, optimizing night sleep quality has the potential of allowing some degree of reduction of anticataplectic medications and a parallel decrease in their side effects.

Behavioral management to optimize night sleep quality comprises those "sleep hygiene" guidelines recommended for all patients with insomnia, but with particular emphasis in narcolepsy for those which improve sleep maintenance, as there is certainly no problem for these patients to fall asleep in the evening! These behaviors include (but are not restricted to): 1) keeping regular bed hours; 2) avoiding rotating shift work; 3) avoiding late evening intense physical activity; 4) avoiding intake of coffee, nicotine or other stimulants, and also alcohol, late in the day; 5) avoiding voluntary sleep in evenings such as napping while watching television; 6) taking a hot bath and/or a warm noncaffeinated beverage before going to bed; 7) using herbal remedies for sleep maintenance; 8) and ensuring evening relaxation coupled with avoidance of intense or stressful activities (18).

Too much should not be expected, however, from interventions which increase night sleep time. Hishikawa et al. (19) permitted ad lib sleep—so-called "sleep satiation"—for up to 20 hours in a group of narcolepsy patients with and without REM-based symptoms. Sleep maintenance insomnia was present only in patients with associated REM-based symptoms and did not improve with extending time in bed, nor did narcolepsy symptoms. A study of Yuchiyama, Mayer and Meier-Ewert (20) involved increasing nocturnal bedtime to 12 hours with daytime sleepiness being measured by MSLT. Night sleep increased from a mean of 417 minutes to 595 minutes; but this three-hour increase was associated with only a modest increase of daytime mean sleep latency from 4.1 to 8.2 minutes. These studies suggest that even

with a quite substantial increase in night sleep time, daytime sleepiness levels remain elevated, and normal waking alertness levels do not return.

However, B. Roth (21) reported that after withdrawal from stimulants a period of ad lib sleep ("sleep therapy") can lead, especially in very severe cases of narcolepsy, to improved symptoms and, over a period of several days, to some decrease in total sleep per 24-hours. Similarly, in temporal isolation studies with ad lib sleep (22) Pollack and Green described an improvement in symptoms associated with a decrease in recorded unintended "daytime" sleep. The efficacy of improving night sleep quality on daytime symptoms in narcolepsy therefore remains unresolved. Moreover, its effect on the ability of patients to resist daytime sleep as tested by the MWT has not been tested using either sleep satiation or temporal isolation approaches.

Procedures which encourage patients to fall back to sleep during a relatively prolonged period of nocturnal wakefulness should also be considered. None have been checked experimentally. These may include getting out of bed and reading, muscle relaxation (such as progressive relaxation therapy), visualizing peaceful scenes, and other approaches.

B. Sleep Paralysis

There is little behaviorally that the patient can do during an episode in order to abort it. Some patients state that a subjective Herculean effort to move can arrest the motor paralysis. However, it is never clear that this did not occur by coincidence at the time the episode was about to cease spontaneously. There is anecdotal evidence for the belief that touching the patient, or even speaking to the patient, can sometimes terminate sleep paralysis. No research has, to our knowledge, been published which objectifies this belief. One publication suggests some efficacy for hypnosis in the control of sleep paralysis (23).

C. Vivid Hypnagogic Hallucinations and REM Nightmares

Vivid hypnagogic hallucinations are typically visual in nature and in essence are the dreams of sleep onset REM periods that characterize about 50% of evening sleep onsets in narcolepsy. These sleep onset dreams may intensify to become hypnagogic REM nightmares. Nightmares are, of course, well documented in narcolepsy for later REM periods during the night. To our knowledge there is no controlled evidence that any behavioral management procedure can alleviate any of these distressing dreamlike and hallucinatory experiences in patients with narcolepsy. However, one case of narcolepsy has been reported in whom lucid dream therapy, in which the disturbing dream content is voluntarily reversed, was of use for nightmares (24).

III. Daytime Symptoms

A. Excessive Daytime Sleepiness and Involuntary Sleep Episodes

Excessive daytime sleepiness is typically the first sign of the onset of narcolepsy and usually precedes the onset of involuntary sleep attacks by a few (1–3) years (11,25). Daly and Yoss (26) based on clinical diagnostic EEGs used the phrase "persistent drowsiness" and provided illustrations of sleep during hyperventilation and photic stimulation, findings that were confirmed by B. Roth (27). Since the discovery of REM

sleep, a daytime propensity to go into both REM and NREM sleep has been well documented using traditional PSG recordings (28), ambulatory monitoring (29) and, of course, the MSLT (30). Daytime recordings are also characterized by marked "waxing and waning" of EEG-defined vigilance levels (31,32) as well as by brief "microsleep" episodes lasting less than 30 seconds (33). The problem appears to reflect insufficiency of daytime arousal levels rather than excessive pressure for sleep (34).

Sleep episodes associated with EDS in narcolepsy vary in duration, sleep stage composition, degree of dissociative features, and other features. Concerning duration, one may encounter a spectrum from very brief lapses or microsleeps (few sec to 30 sec), longer unintended sleep "attacks" or voluntary sleeps (naps) lasting minutes to dozens of minutes, to very long duration episodes of full sleep or of dissociated sleep states some of which have partial awareness, and which are often described as amnesic automatisms. Recordings during the latter have never been published but physiologically they probably consist of long periods of partial sleep states, waxing and waning patterns, or of repeated "microsleeps."

It is widely recognized that patients may usefully adopt a number of behavioral strategies to reduce drowsiness and the probability of falling asleep. Avoidance of hypnogenic situations such sedentary or other inactive behaviors, boredom, overly warm environments, and slow rhythmic stimulation can be helpful. Consciously increasing the level of stimulation is also very effective and is widely used. This includes such approaches as standing rather than sitting, moving as much as possible, increasing background noise levels at work or home, going outside where there are more stimuli, seeking a cooler environment and other strategies mentioned later. It is evident that patients with EDS are much more able to do self-paced activities than those which are externally paced. No behavioral approach has been proposed, let alone been shown to be effective, for amnesic automatic behaviors.

Although in normal persons it is well documented that exposure to bright light has both an alerting effect and a circadian phase setting effect, an attempt to reduce sleepiness in narcolepsy using light therapy (500 lux for 4 hours a day) was unsuccessful (35). However, the level of light exposure in that study is well below that of normal outside levels and the use of more intense light might well prove beneficial.

Scheduled Naps and EDS

Napping is one of the few approaches for which strong objective evidence of efficacy in narcolepsy exists. Billiard (36) first reported improvement in reaction time measures with napping in narcoleptics and noted a greater improvement for daytime NREM, compared to REM, sleep. Godbout and Montplaisir (37) assessed the effects on the Wilkinson and Houghton (38) 10-minute four-choice serial reaction time test just after MSLT naps versus testing at the same hours on days when no naps were taken. They showed that five 20-minute scheduled naps produced a tendency for shortened mean RTs and reduced the number of long "gaps" (RTs $>$1000 msec), which are believed to reflect "lapses" or microsleeps. Guilleminault et al. (39) showed in unmedicated patients that two 15 minute naps scheduled at 12:30 hours and 17:00 hours improved sleep latency on the Maintenance of Wakefulness Test (MWT). This improvement effect on MWT was confirmed for narcoleptic subjects on stimulant medication supplemented by two naps a day (40).

A different approach used by Mullington and Broughton (41) was to determine in patients off stimulant medication, while keeping total sleep per 24-hours constant, whether daytime performance would benefit best from one of three sleep/wake schedules: all sleep being taken at night (no naps); a reduction of night sleep (by 25% of the total) complemented by a single long nap of the duration of the reduction and centered 180° after the mid-point of night sleep (i.e., at the time of the normal afternoon "nap zone"); or an identical reduction of night sleep complimented by five brief naps equidistantly distributed across the wake period (Fig. 1). These studies established several findings for the serial four-choice reaction test (Fig. 2). With no napping there was deterioration in performance after awakening that was maximal in the mid-afternoon and improved later in the day. The single long afternoon nap was effective in reversing the performance deficit for most of the rest of the day. Multiple short naps produced a more sustained level of performance across the entire daytime. These results combined with those of sleep latency studies showing that the effect of a 20 minute MSLT nap on improving alertness is quite short-lived and lasts as little as a half hour (42), indicate that short naps need to be repeated to sustain alertness over time, whereas long naps have a longer duration effect.

Mullington and Broughton also found that unintended sleeps occurred about one to two hours before the normal transient mid-afternoon "nap zone" confirming earlier reports using ambulatory monitoring (43), temporal isolation studies (22), and ultrashort sleep schedules (44). As emphasized by Garma and Marchand (6) this would suggest that a single nap would be best timed somewhat earlier in the daytime, typically around noon, rather than in the mid-afternoon nap zone of normal subjects. Mullington

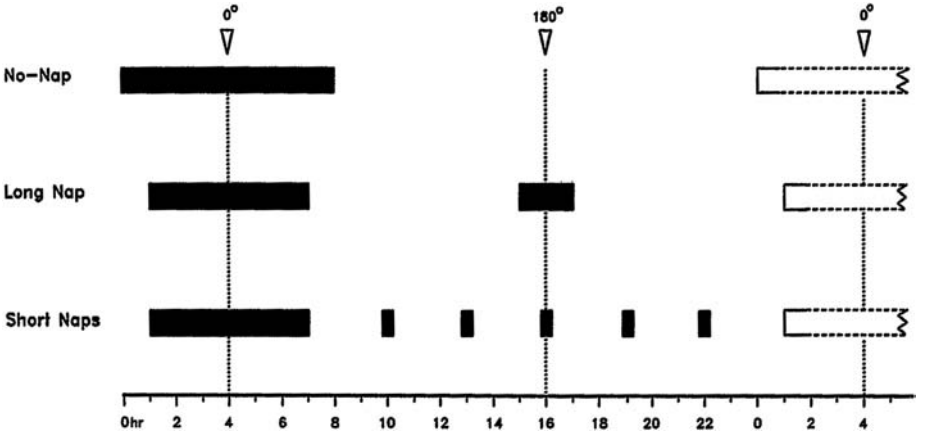


Figure 1 Schematic representation of the sleep/wake schedules followed by narcoleptic subjects during no-nap, single long nap, and multiple short nap conditions. The 24-hour sleep total was kept constant with, in the nap conditions, 75% of sleep taken in the nocturnal and 25% in the naps. The midpoint of nocturnal sleep was also constant across conditions and represents 0°. The midpoint of the long nap and the third short nap are 180° out of phase, with the short naps equidistant across the daytime period. Total 24-hour sleep was based on sleep logs and actigraphic monitoring. The figure represents a schedule in a hypothetical eight-hour sleeper with a nocturnal midpoint at 04:00 hours. *Source*: From Ref. 41.

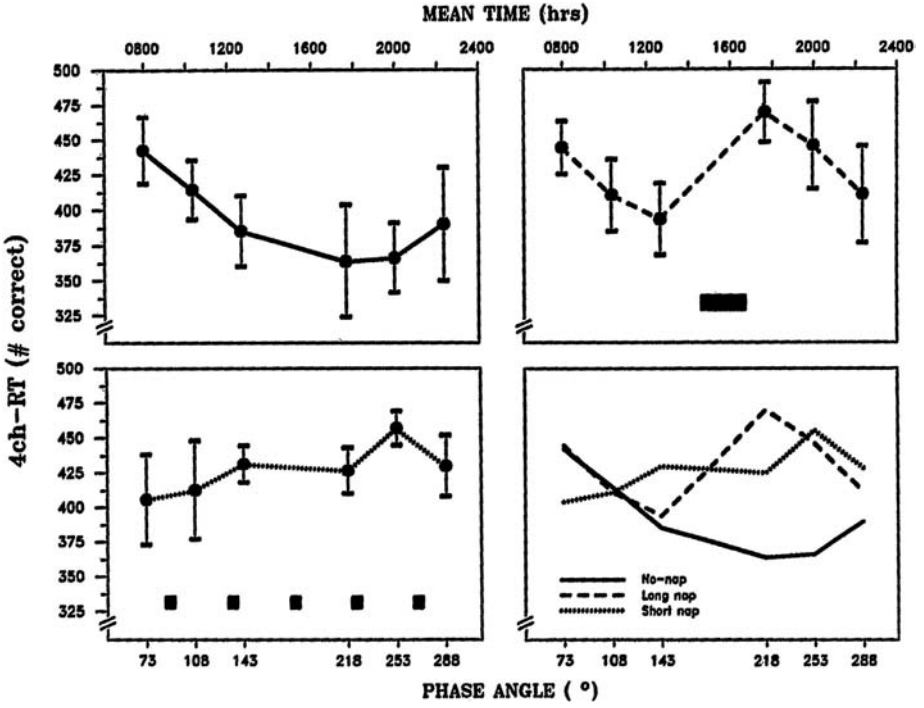


Figure 2 Performance on the serial four-choice reaction time test for all three sleep/wake schedules. No nap condition is in upper left, single nap condition in upper right, multiple nap condition in lower left with superimposed means on lower right. Number of correct responses (means, SEs) are plotted as a function of sleep/wake schedule in degrees (*below*) and time-of-day (*above*). *Source*: From Ref. 41.

and Broughton also found that the earlier belief that naps in narcolepsy are highly refreshing and do not involve sleep inertia is incorrect (45). Although the long nap did not induce sleep inertia, postawakening performance decrement was present for testing immediately after the short naps and could last up to 20 minutes.

Rogers et al. (46) compared three different sleep/wake schedules as an adjunct to stimulant medication for reducing daytime drowsiness and day sleep measured by ambulatory recording. Patients followed one of three schedules for a two week period: their habitual somewhat irregular night sleep patterns supplemented by two 15 minute naps per day, absolutely regular hours of night sleep with no naps, and regular night sleep plus the two naps. The best response (minimal symptoms and least unscheduled daytime sleep) occurred when both night sleep was regularized and naps were taken. The main predictor of degree of improvement was the pretreatment level of sleepiness with sleepier patients responding best to these behavioral interventions.

The ability to take naps varies, of course, with the patient's occupational or educational situation and with the degree of cooperation and understanding of others. Given our current knowledge, it appears that a single prolonged nap, if possible,

would best lead to sustained benefits for three to five hours and that this nap should be taken around noon. This approach may be best suited to the self-employed who have more control over their time. On the other hand, if the workload is evenly distributed across the day, short naps will lead to a more even performance. Short naps are also easier to schedule into a workday and are often more acceptable to employers. As real life examples, one of the subjects in the project of Mullington and Broughton (41) had negotiated with her employer to take multiple (usually 3) naps across the day, whereas a university professor had the habit of going home for lunch, taking a two-hour nap and returning to work. Both believed their work was best suited to, and benefited from, their quite different schedules.

B. Symptoms Secondary to EDS and Sleep Episodes

EDS frequently produces secondary symptoms that, at least to some extent, can also be alleviated by behavioral interventions. These include poor performance, problems in memory and attention, and ocular symptoms.

Performance Problems

The performance measures impaired in narcolepsy are similar to those of sleep deprived normal subjects, although the degree of impact is characteristically much greater. Reaction times are slow, variability of response is higher, there is an increase of unusually long responses ("gaps" defined as RTs over a particular level, typically >1000 msec and which apparently reflect brief lapses of attention or of awareness) and, above all, there is a marked inability to sustain performance levels over time on task (usually referred to as "fatigability"). The latter occurs rapidly, as it is already evident in the second half of a 10 minute choice reaction time task (37) and is progressive across a 30 minute simulated driving task (47). Patients with narcolepsy usually do not make more errors, as they trade off slowness of response for accuracy. Unlike boring brief reaction time tests, short tasks that are highly challenging can be performed at normal levels, as patients with narcolepsy are able to sustain alert wakefulness for such tasks (31). Extreme poor performance exists for very prolonged signal detection "vigilance" tasks such as the one-hour Wilkinson auditory vigilance test (31).

Memory Problems

Memory complaints of narcoleptics were described by Daniels in 1934 (48). An international life-effects study (3) found that 48.9% of patients with narcolepsy-cataplexy reported memory problems (mainly of short term type) that they attributed to their disease, mainly to EDS. Formal neuropsychological testing (49,50), however, has not confirmed a significant memory impairment in untreated or even treated patients. This negative result appears to reflect the ability of narcoleptics to rally their level of alertness in a challenging laboratory situation to perform at normal levels, whereas under normal living conditions they experience much somnolence and so are unable to properly attend to, and encode, events into memory.

Attentional Problems

Application of the Sternberg test of attention, a complex choice reaction time test with various levels of memory load, showed that narcoleptics performed significantly slower

than controls in complex reaction times but without any difference in error rate. The findings suggest a perceptual-encoding deficit (51). Rieger and colleagues (52) assessed tests of focused attention, divided attention and attentional flexibility complemented by a vigilance-type task to determine the capacity to maintain tonic arousal. No differences between controls and patients with narcolepsy were present for either focused attention or divided attention, but narcoleptics did show a deficit in attentional flexibility and in overall attentional control. As in many studies, a good error rate was achieved at the cost of a lower rate of information processing. The vigilance type choice reaction test also confirmed earlier studies (31,37) showing inability to sustain tonic arousal (alertness) over time.

What can behavioral management contribute to minimize these cognitive problems of patients with narcolepsy? First, as the slowed information processing and attentional problems appear directly attributable to sleepiness, this re-enforces the desirability of the patient adopting the various strategies noted earlier to optimize daytime alertness. Secondly, because alertness/sleepiness shows significant variability across the circadian day, important tasks requiring efficiency of information processing or needing memorization should be programmed for periods of higher alertness. The heightened fatigability of performance while on task would indicate that patients should have limited durations on demanding tasks and take frequent breaks. Memory problems can be reduced by keeping lists and by having reminders of things to do, such as using alarms. Patients should be reassured that these problems do not signal dementia and that, with better control of sleepiness, their struggles with cognition will be lessened.

Visual Problems

Visual problems at times reported by patients with narcolepsy include lid drooping, ocular fatigue, defocusing, and double vision. They are particularly challenged by situations where both visual work and sustained attention are involved such as reading. Consequently, prolonged visual tasks including reading and watching television should be minimized.

C. Cataplexy

Behavioral interventions can help minimize the probability of attacks of cataplexy. It has been clearly shown that sleep disruption from stress, night shift work and other factors can increase the frequency of daytime cataplexy. Factors that increase EDS and facilitate the elicitation of cataplexy are therefore to be minimized. Avoidance of the triggers that elicit individual attacks of cataplexy such as jokes, or frightening situations, is particularly helpful. Many patients develop a flat unexcitable affect as a defense against cataplexy. One author has reported a positive response of cataplexy to hypnosis (53), but no controlled studies have been published on this approach.

IV. Psychiatric Problems

Although there is no evidence that narcolepsy is psychological in origin, like most chronic incurable diseases, and perhaps more-so than many, it does have a very

major psychological impact and imposes very significant challenges for adaptation. There is no evidence that narcolepsy is associated with any particular personality type diagnosis, although features of insecurity, loss of confidence, apprehension, and anxiety are common (54–56).

Depression is a frequent complaint of patients with narcolepsy, an association that was emphasized early by Roth and Nevsimalova (57) and confirmed in an international life effects study (3). The possibility of a sleep-related pathophysiological link between narcolepsy and endogenous depression has been suggested (58,59) because in both conditions REM sleep occurs prematurely after sleep onset. However, Vandepulte and Weerd (60) have shown that depressive symptoms on the Beck scale are less frequent in narcolepsy than in a number of other sleep disorders including insufficient sleep, psychophysiological insomnia, restless legs syndrome, obstructive sleep apnea, and even delayed sleep phase syndrome. Moreover, Vourdas and collaborators (61) found that ICD-criteria diagnoses of clinical forms of depression (versus subjective complaints) are no more frequent in narcolepsy than in controls. Behavioral approaches can be employed to improve mood in order to mitigate the intensity and effects of depressive symptoms. Psychotherapy may also, of course, be useful (62), as may cognitive therapy.

Hallucinations are frequently marked in narcolepsy and may lead to diagnostic confusion with schizoprenia. However, there are differences in three respects. In narcolepsy the hallucinations mainly occur during drowsiness rather than in a fully alert (and often excessively alert) state as in schizophrenia. The visual sensory modality is mainly involved in narcolepsy rather than the auditory and at times olfactory ones, as in schizophrenia. Thirdly, the thought disorder so prominent in schizophrenia is absent in narcolepsy. Nevertheless, some overlap may occur and rare patients may have both diseases. Voudras et al. (61) have shown that, if one discounts patients with amphetamine-induced psychosis, the incidence of schizophrenia in patients with narcolepsy is not greater than that in the general public. Douglass and collaborators (62) have reported cases of apparent schizophrenia that turned out to have narcolepsy. As noted above, there are no known helpful behavioral interventions for the hallucinations of narcolepsy.

V. Obesity and Nutrition

Since the discovery of the absence of hypocretin 1 (orexin A) neurons in the hypothalamus of narcoleptic dogs (63) and the very low or absent levels of hypocretin in the CSF of the great majority of patients with narcolepsy-cataplexy (64), strong interest has re-emerged in the area of obesity and narcolepsy. This reflects the role of this polypeptide in the regulation of food intake and energy homeostasis (65), thereby raising the possibility of a common biological substrate.

Narcoleptics patients have been reported to snack more often, more frequently experience sleepiness after meals, especially large ones, and more often crave "sweets" (66). However these findings could not be confirmed in the Pollak and Green study of feeding behavior of narcoleptics under temporal isolation conditions (22). An increase in body mass index has also been reported (67), sleepiness level can be very sensitive to sugar intake (68), and type-2 diabetes mellitus associated with increased body mass is more frequent (69).

Based on this knowledge, the following behavioral interventions would appear worth recommending. Overweight patients should be counseled strongly to lose weight through a decrease in caloric intake combined with increased exercise. It is particularly difficult for patients with EDS to motivate themselves to be more physically active. But the resulting increased levels of alertness are a strong reinforcing influence. Joining an exercise club and committing to an exercise program are very helpful. Very large meals should be avoided, as should foods to which the patient is sensitive and, wherever possible, also alcohol.

VI. Psychosocial Impact and Behavioral Interventions

The psychosocial impact of narcolepsy is profound, broad and lasts for the rest of the patient's life. It is reviewed in a separate chapter and this section is restricted to the behavioral changes which can help alleviate symptoms, with most suggestions coming from patients (8,9).

Problems in education may be alleviated to some extent by behavioral means. Patients can sit at the front of the class to maximize stimulation. Sleepiness may also be improved by moving as much as possible in one's seat without distracting others, choosing an uncomfortable seat or other forms of stimulation, as well as by taking short naps during break periods without classes. Homework should be concentrated into periods of maximum alertness. With the permission of the student, teachers should be made aware of the diagnosis and educated as to its possible impact in class.

Work problems should be minimized using all the recommended maneuvers to increase alertness. It may be necessary to reveal the diagnosis to the employer although many patients are concerned about possible negative consequences including possible job loss. Sympathetic employers should be given information about the condition. Negotiation of times for naps or of being let off rotating shift work may be necessary. Recreational problems may be helped by scheduling activities for periods when patients know they are most alert, and will be more inclined to participate and to commit to group activities.

Driving problems can be minimized using behavioral strategies in persons with narcolepsy who meet local guidelines for licensing. Some patients simply do not drive long distances or drive at night. Patients use many approaches to improve their alertness. They frequently take extra stimulant medication before driving. Patients often have warning sensations before they are at real risk to fall asleep. They will then pull over, stop and walk around or take a caffeinated beverage at a roadside café, and return to the wheel when feeling more alert. We have all encountered patients with narcolepsy who on medication meet driving license regulations, adopt behaviors to minimize the probability of sleepiness at the wheel, and have no reported accidents. To date there is no widely accepted basis on which to permit driving or not.

The negative impact on self-esteem can be reduced by the health care worker taking the time to clarify to the patient and others that he or she is not responsible for having the disease, or for its effects. Low self-esteem can also be improved by having the patient embark on a program to be as productive and as positive as possible. The best behavioral counterfoil to the pressures on interpersonal relationships would appear to be education of the partners, friends, coworkers, employers and others

interacting with the patient. No behavioral interventions have been described as helpful for the stressful area of sexual dysfunction. However, if a regular circadian pattern of increased sexual drive or, for males, in erectile capacity is evident, intimacy would best be scheduled for the time of day when the probability of fulfillment is highest.

Patients with narcolepsy not infrequently report a variety of problems with medication and, sad to say, often do not show reasonable compliance for drugs that will help them (70). Forgetting to take medication is common and can be helped by alarms, pill organizer boxes, spousal reminders and other support. Travel with regulated drugs can lead to problems crossing borders. These can be minimized either by supplying a physician's letter or carrying a card indicating the reason for carrying stimulant medication. There are, unfortunately, quite frequent problems with pharmacies in filling prescriptions due, in part it appears, to concerns of pharmacists about the prescribed stimulants ending up as street drugs. The filling of prescriptions for stimulants in pharmacies can be facilitated by the physician writing "for narcolepsy" on the prescription. Contrary to worries about potential abuse of stimulant medication, most patients with narcolepsy use less medication than is prescribed (70). Discussion with patients about compliance is particularly important with respect to driving.

VII. Coexistent Sleep Disorders

It is now well documented that there is an increased association of sleep apnea (71) and of REM sleep behavior disorder (72,73) in narcolepsy. Not treating these coexistent disorders makes the control of narcolepsy symptoms that much more difficult. Severity of obstructive sleep apnea may be reduced by behavioral interventions. These include weight reduction in overweight patients with OSA and narcolepsy, and positional therapy (such as a tennis ball sewn into the back of pajamas) in patients in whom events are greatly increased while sleeping supine. Unfortunately, there are no known behavioral interventions helpful for RBD.

VIII. Can the Risk of Developing Narcolepsy be Minimized by Behavioral Intervention?

Michell and Dement (74) first suggested that there exists an increased incidence of either irregular sleep or sleep deprivation before the onset of the symptoms in narcolepsy. This was later confirmed and quantified in the life effects study of Broughton et al. (3). The discovery of the HLA-DR2 association was soon followed by the proposal of Langdon and colleagues (75) that narcolepsy is an autoimmune disease in which stresses on the sleep systems lead to altered immune function with an autoimmune response impacting areas of the brain involved in sleep/wake control. Attempts were made to define other possible inducers of narcolepsy in genetically predisposed persons. Some evidence for an association with streptococcal infections was reported (76,77). More recently, the Montpellier group has examined the frequency of stressful life events in the year immediately prior to the onset of narcolepsy and confirmed that it is elevated (78).

The presence of prior poor sleep and the appearance of the disease soon after stressful life events, plus the exceptional cases of reversal of the narcolepsy symptoms

with sleep normalization at the illness onset, together support the possibility that early behavioral interventions can be particularly helpful. Children, adolescents and young adults from families with a history of narcolepsy should be strongly counseled to avoid significant sleep deprivation whether self-imposed, externally imposed, or relating to stress, to keep regular bedtime hours and, for those of working age, to refuse jobs involving rotating shift work.

IX. Patient and Family Education and Counseling

Widespread ignorance still exists about narcolepsy and there is a obligation for the caregiver to provide information or indicate sources of information to patients, family and, at times, employers. Patients, family, and others must become aware of the impact of narcolepsy on the many dimensions of daily life, that no cure yet exists for the disease and that, once present, narcolepsy is a life-long burden. Time must be taken to discuss the symptoms with the patient and to some extent to explain their mechanisms. Patients should be told that even the best therapeutic regimens currently available today cannot be expected to lead to total symptom resolution, particularly in respect to daytime sleepiness. Letters or phone calls to employers may be necessary to ensure scheduled naps at work or to release a patient from rotating shift work. Employers can also be very helpful by moving employees with narcolepsy into areas where sustained alertness over long periods is not involved and where the patient can determine when to accomplish the task rather than having to function in an externally-paced demand situation. The physician and other health care providers must be proactive in helping the patient adapt to this often disabling disease.

Genetic counseling may be needed. Persons with narcolepsy are understandably concerned about the increased risk of a child developing narcolepsy. Patients should be reassured and told that even although the risk of narcolepsy in their child is some 40 times that for the general population, it is still very low. It may be helpful to have the child HLA typed. If a child is negative for the DQB1-*0602 allele, the parent can be further reassured that the risk of occurrence is extremely low.

Useful patient education materials are available in North America from the National Sleep Foundation, the American Narcolepsy Network and the Canadian Sleep Society. Similar organizations exist in many countries. Local patient support groups are often available where persons with narcolepsy may exchange experiences and share behavioral strategies that they have found useful. Internet resources concerning the disease are a very helpful tool for self-education, but patients should be referred to reliable sources to avoid misinformation.

X. Medical Education

Physicians and other health care workers in medical centers with an educational mandate should make forceful demands to increase the time students and medical residents are exposed to lectures on sleep and its disorders including narcolepsy (79). There is still great ignorance in the medical profession about narcolepsy. Misdiagnoses of narcolepsy, mainly with other causes of EDS, are still far too common. The interval between symptom appearance and diagnosis remains unacceptably long. This interval,

which a recent study found to have a mean of 10.5 years, depends in part on the nature of the presenting symptoms, being shortest when clear-cut cataplexy is present (80). A recent study by Kryger and colleagues (81) found a decreasing correct diagnosis rate in the year prior to a sleep center confirmed diagnosis of narcolepsy by neurologists (55% detection rate), internists (23%), general practitioners (21%), psychiatrists (11%), and pediatricians (0%).

In summary, although behavioral interventions on the symptoms and consequences of narcolepsy are often neglected, a compassionate health care worker will make a conscientious effort with each patient to ensure optimum care and support. Behavioral strategies can be used to benefit many areas of function: minimization of symptoms, reduction of impact on performance and amelioration of psychosocial effects. Educational initiatives must be pursued to ensure accurate information for patients, employers, physicians and other health care personnel. While awaiting development of a cure for this frequently debilitating disease, further research is paramount into its behavioral management as well as its pharmacotherapy. Many apparently or potentially helpful behavioral strategies still await controlled study to assess their efficacy.

References

1. Parkes, JD. Sleep and Its Disorders. London, Philadelphia, Toronto: WB Saunders 1985.
2. Broughton R, Valley V, Aguirre M, Roberts J, Suwalski W, Dunham W. Excessive daytime sleepiness and the pathophysiology of narcolepsy: a laboratory perspective. Sleep 1986; 9:205–215.
3. Broughton R, Ghanem Q, Hishikawa Y, Sugita Y, Nevsimalova S, Roth B. Life effects of narcolepsy in 180 patients from North America, Asia and Europe compared to matched controls. Can J Neurol Sci 1981; 8:299–304.
4. Broughton R, Nevsimalova S, Roth B. The socio-economic effects of idiopathic hypersomnia - comparison with controls and with compound narcoleptics. In: Popoviciu L, Asgian B, Badiu G, eds. Sleep 1978. Basel: Karger, 1980:229–233.
5. Beusterien KM, Rogers AE, Walsleben JA, Emsellem HA, Reblando JA, Wang L, Goswami M, Steinwald B. Health-related quality of life effects of modafinil for treatment of narcolepsy. Sleep 1999; 22: 757–765.
6. Garma L, Marchand F. Non-pharmacological approaches to the treatment of narcolepsy. Sleep 1994; 17:S97–S102.
7. Rogers AE, Mullington J. The symptomatic management of narcolepsy. In: ML Perlis, KL Lichstein, eds. Treating Sleep Disorders: Principles and Practice of Behavioral Sleep Medicine. Hoboken, NJ: John Wiley & Son, 2003:118–135.
8. Rogers AE. Problems and coping strategies identified by narcoleptic patients. J Neurosurgical Nursing 1984; 16:326–334.
9. Bruck D, Broughton R. Achieving control over sleepiness in narcolepsy. Australian J. Primary Health 2001; 7:16–24.
10. Daniels E, King MA, Smith IE, Shneerson JM. Health-related quality of life in narcolepsy. J Sleep Res 2001; 10:75–81.
11. Billiard M, Besset A, Cadilhac J. The clinical and polygraphic development of narcolepsy. In: C Guilleminault, E Lugaresi, eds. Sleep/Wake Disorders: Natural History, Epidemiology and Long-Term Evolution. New York: Raven 1983:171–186.
12. Montplaisir J, Godbout R. Nocturnal sleep of narcoleptic patients: revisited. Sleep 1986; 9:159–161.
13. Burton SA, Eastman CI, Kravitz HM. Factors relating to excessive daytime sleepiness in the narcolepsy syndrome. Sleep Res 1988; 17:154.
14. Broughton R, Dunham W, Weisskopf M, Rivers M. Night sleep does not predict day sleep in narcolepsy. Electroencephalogr Clin Neurophysiol 1994; 91:67–70.

15. Ferrans CE, Cohen FL, Smith KM. The quality of life of persons with narcolepsy. Loss, Grief and Care 1992; 5:23–32.
16. Yoss RE, Daly DD. Narcolepsy. Med Clin North Am 1960; 40:954–958.
17. Chen SY, Clift SJ, Dahlitz MJ, Dunn G, Parkes JD. Treatment of the narcoleptic syndrome: self assessment of the action of dexamphetamine and clomipramine. J Sleep Res 1995; 4:113–118.
18. Bergstrom DL, Keller C. Narcolepsy: pathogenesis and nursing care. J Neurosci Nurs 1992; 24: 153–157.
19. Hishikawa Y, Wakamatsu H, Furuya E, Sugita Y, Masaoka S, Kaneda H, Sato M, Nan'no H, Kaneko Z. Sleep satiation in narcoleptic patients. Electroencephalogr Clin Neurophysiol 1976; 41:1–18.
20. Yuchiyama MG, Mayer G, Meier-Ewert K. Differential effects of extended sleep in narcoleptics patients. Electroenceph Clin Neurophysiol 1994; 91:212–218.
21. Roth B. Narcolepsy and Hypersomnia. Basel: Karger 1980:190.
22. Pollak CP, Green J. Eating and its relationships with subjective alertness and sleep in narcoleptic subjects living without temporal cues. Sleep 1980; 13:467–478.
23. Nardi TJ. Treating sleep paralysis with hypnosis. Int J Clin Exp Hypn 1981; 9:358–365.
24. Brulowski A. The role of lucid dreaming in the treatment of narcolepsy and nightmares: a case study. Sleep Res 1987; 16:319.
25. Kales A, Cadieux RJ, Soldatos CR, Bixler EO, Schweitzer PK, Prey WT, Vela-Bueno A. Narcolepsy-cataplexy. 1. Clinical and electrophysiologic characteristics. Arch Neurol 1982; 39:164–182.
26. Daly DD, Yoss RE. Electroencephalogram in narcolepsy. Electroencephalogr Clin Neurophysiol 1957; 9:109–120.
27. Roth B. L'EEG dans la narcolepsie-cataplexie. Electroencephalogr Clin Neurophysiol 1964; 16:170–190.
28. Billiard M, Quera Salva M, de Koninck J, Besset A, Touchon J, Cahilhac J. Daytime sleep characteristics and night sleep in the narcoleptic patient. Sleep 1986; 9:167–174.
29. Broughton R, Dunham W, Newman J, Lutley K, Duschesne P, Rivers M. Ambulatory 24 hour sleep-wake monitoring in narcolepsy-cataplexy compared to matched controls. Electroencephalogr Clin Neurophysiol 1988; 70:473–481.
30. Richardson GS, Carskadon MA, Flagg W, Dement W, Mitler M. Excessive daytime sleepiness in man: sleep latency measurement in narcoleptic and control subjects. Electroencephalogr Clin Neurophysiol 1978; 45:621–627.
31. Valley V, Broughton R. Day-time performance deficits and physiological vigilance in untreated patients with narcolepsy- cataplexy compared to controls. Rev EEG Neurophysiol (Paris), 1981; 11:133–139.
32. Valley V, Broughton R. The physiological (EEG) nature of drowsiness and its relation to performance deficits in narcoleptics. Electroencephalogr Clin Neurophysiol 1983; 55:243–51.
33. Guilleminault C, Billiard M, Montplaisir J, Dement WC. Altered states of consciousness in disorders of daytime sleepiness. J Neurol Sci 1975; 26:377–393.
34. Broughton R, Krupa S, Boucher B, Rivers M, Mullington J. Impaired circadian waking arousal in narcolepsy-cataplexy. Sleep Research Online 1998; 1:159–165 (http://sro.org/1998/broughton/159).
35. Hajek M, Meier-Ewert K, Wiz-Justice A, Tobler I, Arendt J, Dick H, Fink G. Bright white light does not improve narcoleptic symptoms. Eur Arch Psychiatr Neurol Sci 1989; 238:203–207.
36. Billiard M. Competition between the two types of sleep and the recuperative function of REM sleep versus NREM sleep in narcoleptics. In: C Guilleminault, WC Dement, P Passouant, eds. Narcolepsy. New York: Spectrum Publications 1976:77–96.
37. Godbout R, Montplaisir J. All-day performance variations in normal and narcoleptic subjects. Sleep 1986; 9:200–204.
38. Wilkinson R, Houghton D. Portable four-choice reaction time test with magnetic memory. Behav Res Meth Instrum 1975; 7:441–446.
39. Guilleminault C, Stoohs R, Clerk A. Daytime somnolence: therapeutic approaches. Neurophysiol Clin 1993; 23:23–33.
40. Rogers AE, Aldrich MS. The effect of regularly scheduled naps on sleep attacks and excessive daytime sleepiness associated with narcolepsy. Nursing Res 1993; 42:111–117.
41. Mullington J, Broughton R. Scheduled naps in the management of daytime sleepiness in narcolepsy-cataplexy. Sleep 1993; 16:444–56.
42. Roehrs T, Zorick F, Wittig R, Paxton C, Sicklesteel J, Roth T. Alerting effects of naps in patients with narcolepsy. Sleep 1986; 9:194–199.
43. Mullington J, Dunham W, Broughton R. Phase timing and duration of naps in narcolepsy-cataplexy: preliminary findings. On: J Horne, ed. Sleep'90. Pontenagel: Bochum, 1990:158–160.

44. Lavie P. REM periodicity under ultrashort sleep/wake cycle in narcoleptic patients. Can J Psychol 1991; 45:85–93.
45. Mullington J, Broughton R. Daytime sleep inertia in narcolepsy-cataplexy. Sleep 1994; 17:69–76.
46. Rogers AE, Aldrich MS, Lin X. A comparison of three different sleep schedules for reducing sleepiness in narcolepsy. Sleep 2001; 24:385–391.
47. George CFP, Bourreau AC, Smiles A. Comparison of simulated driving performance in narcolepsy and sleep apnea patients. Sleep 1996; 19:711–717.
48. Daniels LE. Narcolepsy Medicine 1934; 13:1–122.
49. Aguirre M, Broughton R, Stuss D. Does memory impairment exist in narcolepsy-cataplexy? J Clin Exp Neuropsychol 1985; 7:14–24.
50. Rogers AE, Rosenberg RS. Tests of memory in narcoleptics. Sleep 1990; 13:42–52.
51. Henry GK. Satz P, Heilbronner RL. Evidence of a perceptual-encoding deficit in narcolepsy. Sleep 2003; 16:123–127.
52. Rieger M, Mayer G, Gauggel S. Attention deficits in patients with narcolepsy. Sleep 2003; 26:36–43.
53. Price R. Hypnotherapy in the control of cataplexy in a narcoleptic subject. Am J Clin Hypn 1981; 29: 201–205.
54. Kales A, Soldatos CR, Bixler EO, Caldwell A, Cadieux RJ, Verrechio JM, Kales JD. Narcolepsy-cataplexy. II. Psychosocial consequences and associated psychopathology. Arch Neurol 1982; 39:169–171.
55. Thorpy M. Current concepts in the etiology, diagnosis and treatment of narcolepsy. Sleep Med 200; 2:5–17.
56. Reinish LW, Devins GM, Shapiro CM. Personality assessment in narcolepsy. Sleep Res 1993; 22:160.
57. Roth B, Nevsimalova S. Depression in narcolepsy and hypersomnia. Schweiz Arch Neurol Neurochir Psychiatr 1975; 116:291–300.
58. Reynolds CF, Christiansen CL, Taksa LS, Coble PA, Kupfer D. Sleep in narcolepsy and depression: does it all look alike? J Nerv Ment Dis 1983; 171:290–295.
59. Hudson JI, Pope HG, Sullivan LE, Waternaux CM, Keck PE, Broughton RJ. Good sleep, bad sleep: a meta-analysis of polysomnographic measures in insomnia, depression, and narcolepsy. Biol Psychiatry 1992; 32:958–975.
60. Vandeputte M, de Weerd A. Sleep disorders and depressive feelings: a global survey with the Beck depression scale. Sleep Med 2003; 4:343–345.
61. Vourdas A, Sheerson JM, Gregory CA, Smith IE, King MA, Morrish E, McKenna PJ. Narcolepsy and psychopathology: is there an association. Sleep Med 2002; 3:353–360.
62. Douglass AB, Hays P, Pazderka F, Russell JM. Florid refractory schizophrenias that turn out to be treatable variants of HLA-associated narcolepsy. J Nerv Ment Dis 1991; 179:12–17.
63. Lin L, Faraco J, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. The sleep disorder of canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999; 98:365–376.
64. Nishino N, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000; 355:39–40.
65. Kukkonedn JP, Holmquist T, Ammoun S, Akerman KE. Functions of the orexinergic/hypocretinergic system. Am J Physiol Cell Physiol 2002; 283:C1567–C1591.
66. Bell IR, Guilleminault C, Dement WC. Questionnaire survey of eating habits of narcoleptics versus controls. Sleep Res 1975; 4:208.
67. Schuld A, Hebebrand J, Geller F, Pöllmächer T. Increased body mass index in patients with narcolepsy. Lancet 2000; 355:39–40.
68. Bruck D, Armstrong S, Coleman G. Sleepiness after glucose in narcolepsy. J Sleep Res 1994; 3:171–179.
69. Honda Y, Doi Y, Ninomiya R, Ninomiya C. Increased frequency of non-insulin-dependent diabetes mellitus among narcoleptic patients. Sleep 1986; 9:254–259.
70. Rogers AE, Aldrich MS, Berrios AM, Rosenberg RS. Compliance with stimulant medication in patients with narcolepsy. Sleep 1997; 20:28–33.
71. Chokroverty S. Sleep apnea in narcolepsy. Sleep 1986; 9:250–253.
72. Mayer G, Meier-Ewert KH. Motor dyscontrol in sleep of narcoleptic patients (a lifelong development?). J Sleep Res 1993; 2:143–148.
73. Schenck CH, Mahowald MW. Motor dyscontrol in narcolepsy: rapid eye movement sleep with atonia and REM sleep behavior disorder. Ann Neurol 1992; 32:3–10.

74. Mitchell SA, Dement WC. Narcolepsy syndromes: antecedent, contiguous and concomitent nocturnal sleep disordering and deprivation. Psychophysiol 1968; 4:398.
75. Langdon N, Lock C, Welsh K, Vergami D, Dorow R, wachet H, Palenschat D, Parkes JD. Immune factors in narcolepsy. Sleep 1986; 9:143–148.
76. Billiard M, Laaberki MF, Reygrobellet C, Seignalet J, Brissaud L, Besset A. Elevated antibodies to streptococcal antigens in narcoleptic subjects. Sleep Res 1989; 18:201.
77. Montplaisir J, Poirier G, Lapierre O, Montplaisir S. Streptococcal antibodies in narcolepsy and idiopathic hypersomnia. Sleep Res 1989; 18:271.
78. Orellana C, Villemin E, Tafti M, Carlander B, Besset A, Billiard M. Life events in the year preceding the onset of narcolepsy. Sleep 1994; 17:S50–S53.
79. Rosen R, Mahowald M, Chesson A, Doghramji K, Goldberg R, Moline M, Millman R, Zammit G, Mark B, Dement WC. The Taskforce 2000 survey on medical education in sleep and sleep disorders. Sleep 1998; 21:235–238.
80. Moorish E, King MA, Smith IE, Shneerson JM. Factors associated with a delay in the diagnosis of narcolepsy. Sleep Med 2004; 4:37–41.
81. Kryger MH, Walid R, Manfreda J. Diagnoses received by narcolepsy patients in the year prior to diagnosis by a sleep specialist. Sleep 2002; 25:36–41.

48
Pharmacology of CNS Stimulants

SEIJI NISHINO and EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

Central Nervous System (CNS) stimulants used in sleep medicine include amphetamine-like compounds (l- and d-amphetamine and methamphetamine, methylphenidate, pemoline), mazindol, modafinil, some antidepressants with stimulant properties (e.g., buproprion) and caffeine. The effects of most of these drugs on wakefulness is primarily mediated via an inhibition of dopamine reuptake/transport and in some cases, via increased dopamine release. An exception is caffeine, a compound with adenosine receptor antagonistic effects. Biogenic amine transporters [for dopamine (DA), norepinephrine (NE), and serotonin] are located at nerve terminals and are important in terminating transmitter action and maintaining transmitter homeostasis. In the past decade, monoamine transporters have been cloned and their molecular mechanisms have been elucidated and genetically engineered mice lacking these molecules (knockout mice) have also become available. In parallel with these discoveries, potent and selective ligands for the DA and NE transporters have been developed. The results of pharmacological studies using these new ligands in canines and knockout mice models suggest the importance of the DA transporter (as opposed to the adrenergic transporter) for the mode of action of amphetamines, amphetamine-like compounds and bupropion on wakefulness. Importantly, however, the various stimulants also have differential effects on dopamine storage (via VMAT inhibition) or release (and in most cases) have effects on other monoaminergic systems. The mode of action of modafinil, a more recent compound used as a first line treatment for excessive daytime sleepiness (EDS), is controversial. Dopamine reuptake inhibition may also be critical in modafinil's primary mechanism of action.

In this chapter, we provide an overview of the pharmacological mechanisms of CNS stimulants and their clinical applications.

II. Amphetamines and Amphetamine-Like Compounds

Amphetamine was first synthesized in 1897, but its stimulant effects were not recognized until 1929 by Alles. In 1935, amphetamine was used for the first time for the treatment of narcolepsy. Narcolepsy was possibly the first condition for which amphetamine was used clinically. It revolutionized therapy for the condition, even though it was not a curative. The piperazine derivative of amphetamine, methylphenidate, was introduced

for the treatment of narcolepsy in 1959 by Yoss and Daly, but both compounds share the similar pharmacological properties.

Amphetamine and methylphenidate are primarily indicated for narcolepsy, idiopathic hypersomnia and attention deficit hyper activity disorder (ADHD). Other therapeutic uses are controversial because of their abuse potential. As a result, amphetamine is classified as a schedule II substance and methylphenidate is classified as a schedule III substance under the Controlled Substances Act of 1970.

Phenylisopropylamine (amphetamine) has a simple chemical structure resembling endogenous catecholamines (Fig. 1). This scaffold forms the template for a wide variety of pharmacologically active substances. Although amphetamine possesses strong central stimulant effects, minor modifications can result in agents having a broad spectrum of effects, including nasal decongestion anorexia, vasoconstriction, antidepressant effects (for the monoamine oxidase inhibitor MAOI, tranylcypromine), or hallucinogenic properties (MDMA [methyllendioxy-methamphetamine] and MDA [methylenedioxyamphetamine]).

The pharmacological effects of most amphetamine derivatives are isomer-specific. D-Amphetamine is for example, a far more potent stimulant than the l-derivative. In EEG studies, d-amphetamine is four times more potent in inducing wakefulness than l-amphetamine (1). Not all effects are stereospecific however. For example, both enantiomers are equipotent at suppressing REM sleep in humans and rats (1) and at producing amphetamine psychosis. The relative effects of the d- and l-isomers of amphetamine on NE and DA transmission may explain some of these differences (for details, see the pharmacology section).

Amphetamine-like compounds, such as methylphenidate, pemoline, and fencamfamin are structurally similar to amphetamines; all compounds include a benzene core with an ethylamine group side chain (Fig. 1). Both methylphenidate and pemoline were commonly used for the treatment of EDS in narcolepsy, but pemoline has been withdrawn from the market in several countries because of liver toxicity (Table 1). The

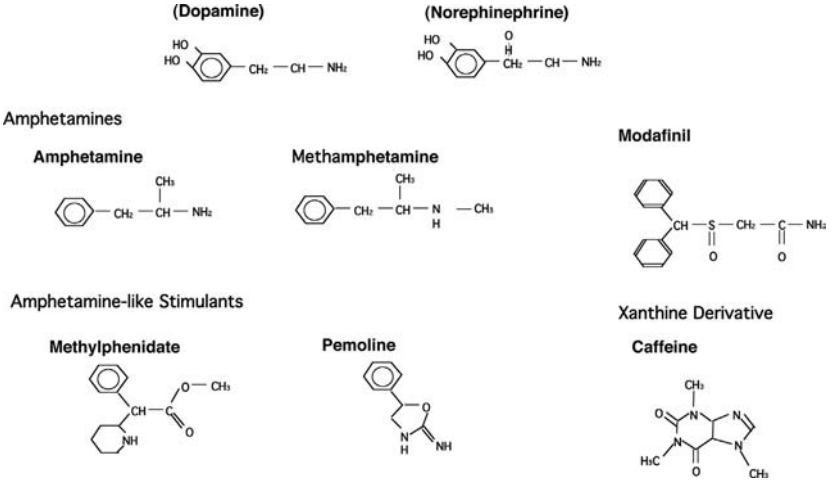


Figure 1 Chemical structures of amphetamine-like stimulants, modafinil and caffeine (a xanthine derivative), as compared to dopamine and norepinephrine.

most commonly used form of methylphenidate is a racemic mixture of both a d- and l-enantiomer, but d-methylphenidate mainly contributes its clinical effects, especially after oral administration. This is due to the fact that l-methylphenidate, but not

Table 1 Commonly Used Pharmacological Compounds for Excessive Daytime Sleepiness

Stimulant compound	Usual daily doses[a]	Side effects/notes
Amphetamine-like CNS stimulants:		
D-amphetamine sulfate	5–60 mg (15, 100 mg)	Irritability, mood changes, headaches, palpitations, tremors, excessive sweating, insomnia
Methamphetamine HCl	5–60 mg (15, 80 mg)	Same as D-amphetamine May have a greater central over peripheral effects than D-amphetamine[b]
Methylphenidate HCl	10–60 mg (30, 100 mg)	Same as amphetamines, Better therapeutic index than D-amphetamine with less reduction of appetite or increase in blood pressure Short duration of action
Pemoline	20–115 mg (37.5, 150 mg)	Less sympathomimetic effect, milder stimulant Slower onset of action, a tendency for drug build-up occasionally produces liver toxicity pemoline is not a controlled substance
DA/NE uptake inhibitor:		
Mazindol	2–6 mg (na)	Weaker CNS stimulant effects, anorexia, dry mouth, irritability, headaches, gastrointestinal symptoms Reported to have less potential for abuse
Other agents for treatment of EDS:		
Modafinil	100–400 mg (na)	No peripheral sympathomimetic action, headaches, nausea Reported to have less potential for abuse
MAO inhibitors with alerting effect:		
Selegiline	5–40 mg (na)	Low abuse potential Partial (10–40%) interconversion to amphetamine
Xanthine derivative		
Caffeine[c]	100–200 mg (na)	Weak stimulant effect, 100 mg of caffeine roughly equivalent to one cup of coffee, palpitations, hypertension

[a]Demonstrated anticataplectic effects in humans.
[b]Dosages recommended by the American Sleep Disorders Association are listed in parenthesis (usual starting dose and maximal dose recommended).
[c]Caffeine can be brought without prescription in the form of tablets (No Doz®, 100 mg; Vivarin® 200 mg caffeine) and is used by many patients with narcolepsy prior to diagnosis. Methamphetamine is reported to have more central effects and may predispose more to amphetamine psychosis. The widespread misuse of methamphetamine has led to severe legal restriction on its manufacture, sale and prescription in many countries. l-amphetamine (dose range, 20–60 mg) is not available in the U.S.A., but probably has no advantage over D-amphetamine in the treatment of narcolepsy (slightly weaker stimulant).
Abbreviations: CNS, central nervous system; DA, dopamine; EDS, excessive daytime sleepiness; MAO, monoamine oxidase; NE, norepinephrine.

d-methylphenidate, undergoes a significant first-pass metabolism (by de-esterification to l-ritalinic acid).

Amphetamines are highly lipid soluble molecules that are well absorbed by the gastrointestinal tract. Peak levels are achieved approximately 2 hours after oral administration, with rapid tissue distribution and brain penetration. Protein binding is highly variable, with an average volume of distribution (Vd) of 5 L/Kg. The effects of amphetamine last approximately 6–10 hours, with a half-life of 16–30 hours. Both hepatic catabolism and renal excretion are involved in the inactivation of amphetamine. Methylphenidate is almost totally absorbed after oral administration, and peak levels are achieved approximately 1–2 hours with rapid tissue distribution and brain penetration, but slow release forms (the peak levels 5–6 hours) are also available. Methylphenidate has low protein binding (15%) and is fairly short acting; the effects last approximately 4 hours, with a half-life of 3 hours. The primary means of clearance is through the urine, in which 90% is excreted.

III. Molecular Targets of Amphetamine Action

The molecular targets mediating amphetamine-like stimulant effects are complex and vary depending of the specific analogue/isomer and the dose administered. Amphetamine per se, increases catecholamine (DA and NE) release and inhibits reuptake. These effects are mediated by specific catecholamine transporters (2) (Fig. 2). The DA transporter [DAT] and the NE transporter [NET], have now been cloned and characterized. The DAT and NET proteins are about 620 amino-acid proteins with 12 putative membrane-spanning regions. Amphetamine derivatives inhibit the uptake and enhances the release of DA, NE or both by interacting these molecules. The DAT and NET normally move DA and NE respectively from the outside to the inside of the cell. This process is sodium-dependent; sodium and chloride bind to the DA/NE transporter to immobilize it at the extracellular surface and to alter the conformation of the DA/NE binding site thereby facilitating substrate binding. Substrate binding allows movement of the carrier to the intracellular surface of the neuronal membrane, driven by the sodium concentration gradients. Interestingly, in the presence of some drugs such as amphetamine, the direction of transport appears to be reversed (Fig. 2). DA and NE are moved from the inside of the cell to the outside through a mechanism called exchange diffusion, which occurs at low doses (1–5 mg/kg) of amphetamine, and this mechanism is involved in enhancement of catecholamine release by amphetamine. A recent in vitro experiment has shown that amphetamine transportation causes an inward current, and intracellular sodium ion becomes more available, thereby enhancing DAT-mediated reverse transport of DA.

At higher doses, other effects are involved. Moderate to high doses of amphetamine (>5 mg/kg) interact with the vascular monoamine transporter 2 (VMAT2) (2). The vesicularization of the monoamines (DA, NE serotonin, and histamine) in the central nervous system is dependent on VMAT2; VMAT2 regulates the size of the vesicular and cytosolic DA pools. Amphetamine is highly lipophilic and easily enters nerve terminals by diffusing across several mechanisms. Amphetamine leads to a diffusion of the native monoamines out of the vesicles into the cytoplasm along a concentration gradient and acts as a physiological VMAT2 antagonist that releases the vascular DA/NE into the cytoplasm. These mechanisms, as well as the reverse

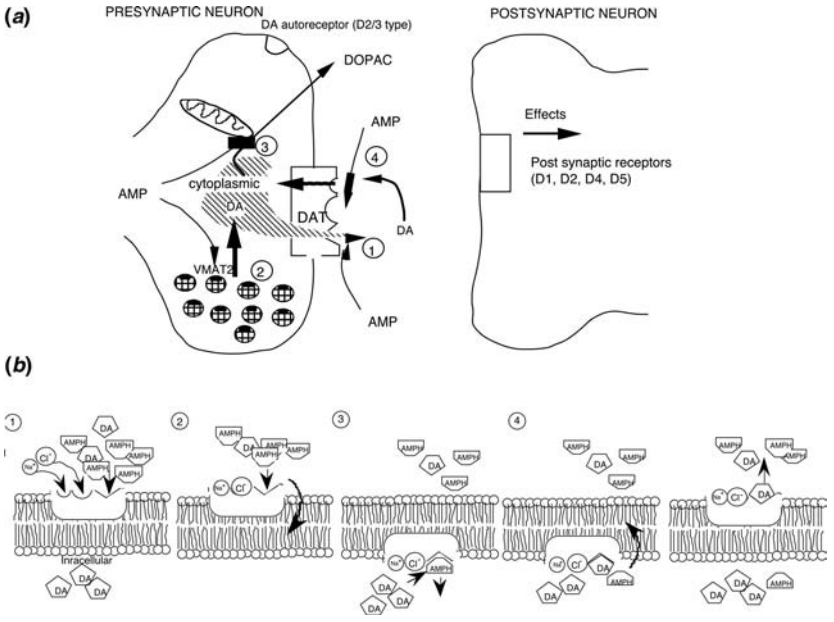


Figure 2 (***a***) Effects of amphetamines at the dopaminergic nerve terminal (***b***) Schematic model of the exchange-diffusion process in relation to the mode of action of amphetamines. (***a***) 1) amphetamine interacts with the dopamine (DA) transporter (DAT) carrier to facilitate DA release from the cytoplasm through an exchange diffusion mechanism. At higher intracellular concentrations, amphetamine also 2) disrupts vesicular storage of DA, and 3) inhibits the monoamine oxidase (MAO). Both these actions increase cytoplasmic DA concentrations. 4) Amphetamine also inhibits DA uptake by virtue of its binding to and transport by the DAT; DOPAC, dihydroxyphenylacetic acid. (***b***) 1) Sodium and chloride bind to the DAT to immobilize it at the extracellular surface. This alters the conformation of the DA binding site on the DAT to facilitate substrate binding. 2) Amphetamine, in competition with extracellular DA, binds to the transporter. Substrate binding allows the movement of the carrier to the intracellular surface of the neuronal membrane, driven by the sodium and amphetamine concentration gradients, resulting in a reversal of the flow of DA uptake. 3) Amphetamine (AMP) dissociates from the transporter, making the binding site available to cytoplasmic DA. 4) DA binding to the transporter enables the movement of the transporter to the extracellular surface of the neuronal membrane, as driven by the favorable DA concentration gradient. 5) DA dissociates from the transporter, making the transporter available for amphetamine, and thus another cycle. *Source*: Adapted from Ref. 2.

transport and the blocking of reuptake of DA/NE by amphetamine, all lead to an increase in NE and DA synaptic concentrations (2). High doses (higher than a clinical dose) of amphetamines are also shown to inhibit monoamine oxidase and prevent catecholamine metabolism.

Various amphetamine derivatives have slightly different effects on all these systems. For example, methylphenidate also binds to the NET and DAT and enhances catecholamine release, but has less effect on the VMAT granular storage site than amphetamine. Similarly, d-amphetamine has proportionally more releasing effect on the DA versus the NE system when compared to l-amphetamine. Of note, other antidepressant

medications acting on catecholamines, including both DA and NE (for example: bupropion or mazindol), tend to exert their actions by simply blocking the reuptake mechanism.

IV. Dopaminergic Neurotransmission and EEG Arousal

How amphetamines and other stimulants increase EEG arousal has been explored using a canine model of the sleep disorder narcolepsy and DAT knockout mice models. Canine narcolepsy is a naturally occurring animal model of the human disorder (3). Similar to human patients, narcoleptic dogs are excessively sleepy (i.e., shorter sleep latency), have fragmented sleep patterns, and display cataplexy (3). Although amphetamine-like compounds are well known to stimulate catecholaminergic transmission, the exact mechanism by which they promote EEG arousal is still uncertain. Stimulation of either or both adrenergic or dopaminergic transmission have been suggested to play a role.

In order to address this question, the effects of ligands specific for the DA (GBR12909, bupropion and amineptine), NE (nisoxetine and desipramine) or both the DA and NE (mazindol and nomifensine) transporters, as well as amphetamine and a non-amphetamine stimulant, modafinil, were studied in narcoleptic and control Dobermans (4). DA uptake inhibitors, such as GBR12909 and bupropion, dose-dependently increased EEG arousal in narcoleptic dogs, while nisoxetine and desipramine (two potent NE uptake inhibitors,) had no effect on EEG arousal at doses which almost completely suppressed REM sleep and cataplexy (4). Most strikingly, the EEG arousal potency of various DA uptake inhibitors correlated tightly with in vitro DA transporter binding affinities (Fig. 3), while a reduction in REM sleep correlated with in vitro NET binding affinities (4). These results strongly suggest that DA uptake inhibition is critical for the EEG arousal effects of these compounds.

Of note, d-amphetamine, has a relatively low DA transporter binding affinity but potently (i.e., need for a low mg/kg dose) promotes alertness (Fig. 3). It is also generally considered to be more efficacious (i.e., can produce more alertness at a higher dose) than pure DAT reuptake inhibitors in promoting wakefulness. However, as described earlier, d-amphetamine not only inhibits DA reuptake, it also enhances DA release (at lower dose by exchange diffusion and at higher dose by antagonistic action against VMAT2) and inhibits monoamine oxidation to prevent DA metabolism. The DA releasing effects of amphetamine are likely to explain the unusually high potency and efficacy of amphetamine in promoting EEG arousal.

In order to further differentiate between the involvement of the DA and NE systems in the mode of action of amphetamine derivatives, the effects of various amphetamine analogs (d-amphetamine, l-amphetamine, and l-methamphetamine) on EEG arousal and their in vivo effects on brain extracellular DA levels in narcoleptic dogs were compared (5). In vitro studies have demonstrated that the potency and selectivity for enhancing release or inhibiting uptake of DA and NE vary between amphetamine analogs and isomers (6). Amphetamine derivatives, thus offer a unique opportunity to study the pharmacological control of alertness in vivo. Hartmann and Cravens previously reported that d-amphetamine is 4 times more potent in inducing EEG arousal than is l-amphetamine, but that both enantiomers are equipotent at suppressing REM sleep in humans and rats (1). Enantiomer-specific effects have also been reported with methamphetamine; l-methamphetamine is much less potent as a stimulant than either d-methamphetamine or d- or l-amphetamine (6). Similarly, in canine

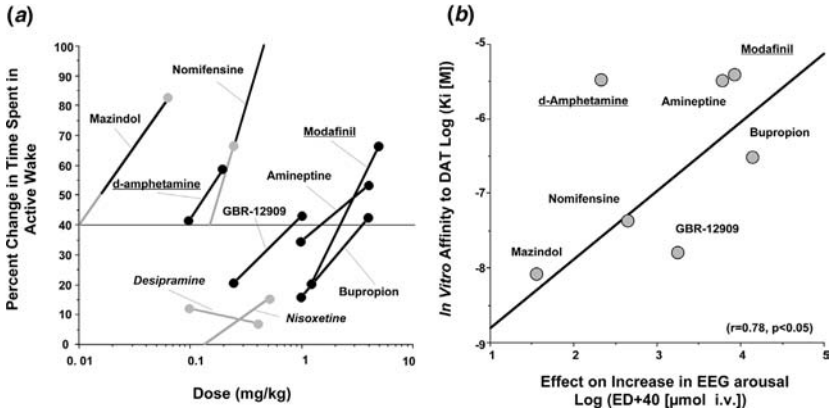


Figure 3 (***a***) Effects of various DA and NE uptake inhibitors and amphetamine-like stimulants on the EEG arousal of narcoleptic dogs and (***b***) correlation between in vivo EEG arousal effects and in vitro DA transporter binding affinities. (***a***) The effects of various compounds on daytime sleepiness was studied using 4 hr daytime polygraphic recordings (10:00–14:00) in 4-5 narcoleptic animals. Two doses were studied for each compound. All DA uptake inhibitors and CNS stimulants dose-dependently increased EEG arousal and reduced SWS when compared to vehicle treatment. In contrast, nisoxetine and desipramine, two potent NE uptake inhibitors, had no significant effect on EEG arousal at doses that completely suppressed cataplexy. Compounds with both adrenergic and dopaminergic effects (nomifensine, mazindol, D-amphetamine) were active on both EEG arousal and cataplexy. The effects of the two doses studied for each stimulant were used to approximate a dose-response curve; the drug dose which increased the time spent in wakefulness by 40% above baseline (vehicle session) was estimated for each compound. The order of potency of the compounds obtained was: mazindol > (amphetamine) > nomifensine > GBR 12,909 > amineptine > (modafinil) > bupropion. (***b***) In vitro DAT binding was performed using [3H]-WIN 35,428 onto canine caudate membranes. Affinity for the various DA uptake inhibitors tested varied widely between 6.5 nM and 3.3 mM. In addition, it was also found that both amphetamine and modafinil have a low, but significant affinity (same range as amineptine) for the DAT. A significant correlation between in vivo and in vitro effects was observed for all 5 DA uptake inhibitors and modafinil. Amphetamine, which had potent EEG arousal effects, has a relatively low DAT binding affinity, suggesting that other mechanisms, most probably monoamine releasing effects or monoamine oxidase inhibition, are also involved. In contrast, there was no significant correlation between in vivo EEG arousal effects and in vitro NE transporter binding affinities for DA and NE uptake inhibitors. These results suggest that presynaptic enhancement of DA transmission is the key pharmacological property mediating the EEG arousal effects of most wake-promoting CNS stimulants. *Soruce*: Adapted from Ref. 4.

narcolepsy, d-amphetamine is 3 times more potent than l-amphetamine, and 12 times more potent than l-methamphetamine in increasing wakefulness and reducing Slow Wave Sleep (5).

To further study what mediates these differences in potency, the effects of these amphetamine derivatives on DA release were examined in freely moving animals using in vivo microdialysis. Amphetamine derivatives (100 μM) were perfused locally for 60 min through the dialysis probe implanted in the caudate of narcoleptic dogs (5). The local perfusion of d-amphetamine raised DA levels 9 times above baseline. L-amphetamine also increased DA levels by up to 7 times, but peak DA release was

only obtained at the end of the 60 min perfusion period. L-methamphetamine did not change DA levels under these conditions. These results suggest that d-amphetamine is more potent than l-amphetamine in increasing caudate DA levels, while l-methamphetamine had the least effect-in agreement with data obtained in other species using same technique (6). NE was also measured in the frontal cortex during perfusion of d-amphetamine, l-amphetamine and l-methamphetamine. Although all compounds increased NE efflux, no significant difference in potency was detected among the three analogs.

The fact that the potency of amphetamine derivatives on EEG arousal correlates with effects on DA efflux in the caudate of narcoleptic dogs further suggest that the enhancement of DA transmission by presynaptic modulation mediates the wake-promoting effects of amphetamine analogs. This result is also consistent with data obtained with DA transporter blockers (see Fig. 3). Considering the fact that other amphetamine-like stimulants (such as methylphenidate and pemoline) also inhibit DA uptake and enhance release of DA, the presynaptic enhancement of DA transmission is likely to be the key pharmacological property mediating wake-promotion for all amphetamines and amphetamine-like stimulants.

The role of the DA system in sleep regulation was further assessed using mice lacking the DAT gene. Consistent with a role of DA in the regulation of wakefulness, these animals have reduced non-REM sleep time and increased wakefulness consolidation (independently from locomotor effects) (7). DAT knock-out mice have also proven to be a powerful tool to help dissect the molecular mechanisms mediating the effects of nonselective monoaminergic compounds. Using these animals, DAT was shown to be involved in mediating locomotor activation after amphetamine and cocaine administration. Indeed, no locomotor stimulation is observed in these mice after cocaine or amphetamine. Interestingly, NET knock-out mice are more sensitive to the locomotor stimulation of amphetamine, suggesting that NET may play a feedback control role on amphetamine-induced dopaminergic effects (8). With regard to sleep, the most striking finding was that DAT knockout mice were completely unresponsive to the wake-promoting effects of methamphetamine, GBR12909 (a selective DAT blocker) and modafinil. These results further confirm the critical role of DAT in mediating the wake-promoting effects of amphetamines and modafinil (Fig. 4) (7) (see also modafinil section). Interestingly, DAT knockout animals were also found to be more sensitive to caffeine (7), suggesting functional interactions between adenosine and DA systems in the control of sleep/wake (see also caffeine/adenosine section).

The VMAT2 gene has been cloned and mice lacking VMAT2 have also been produced (9). Although homozygous VMAT2 knockout mice (VMAT2$-/-$) are lethal, heterozygous animals (VMAT2$+/-$) survive and express a 50% reduction of VMAT2 concentration. VMAT2 $+/-$ have a significant deficit of DA transmission but normal noradrenergic and serotonergic neurotransmission (9). VMAT2 heterozygous knockout mice are supersensitive to amphetamine locomotor stimulation, but have attenuated amphetamine-behavioral reward (9). Recent results in our laboratory suggest that methamphetamine and modafinil similarly promote wakefulness in both VMAT2$+/-$ and VMAT2$+/+$ animals. The results contrast with the absence of any wake promoting effects with these drugs in DAT knockout mice, and suggests that VMAT blockade by amphetamine is not essential for its wake-promoting effect.

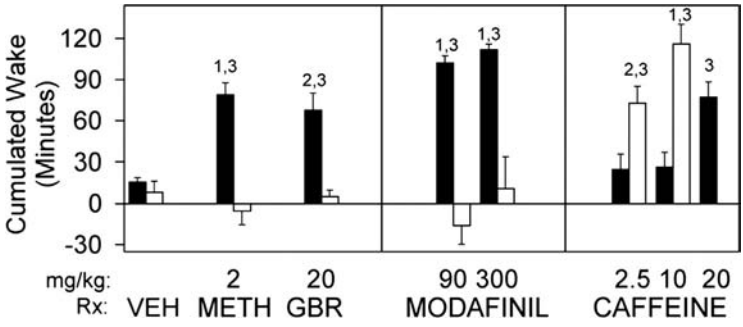


Figure 4 Response to wake-promoting therapeutics in DAT knock-out (open bars) and wild-type (filled bars) mice. Data is reported as the cumulative change in time awake (mean + SEM) 5 hr after treatment relative to corresponding baseline 24 hr earlier. Two-way ANOVA indicated significant genotype_x treatment interaction in comparisons of individual pharmacological treatments versus vehicle (p, 0.05, all treatments). VEH, 0.25% methylcellulose vehicle; METH, methamphetamine; GBR, GBR12909. 1p, 0.001; 2p, 0.025 between groups; 3p, 0.002 relative to vehicle; Student's t test (sample size: 6–14 for each session). *Source*: Adapted from Ref. 7.

V. Anatomical Substrates of Dopaminergic Effects

Anatomical studies have demonstrated two major subdivisions of the ascending DA projections from mesencephalic DA nuclei (VTA, SN and retrorubral [A8]): (1) The mesostriatal system originates in the SN and retrorubral nucleus and terminates in the dorsal striatum (principally the caudate and putamen) (10). (2) The mesolimbocortical DA system consists of the mesocortical and mesolimbic DA systems. The mesocortical system originates in the VTA and the medial SN and terminates in the limbic cortex (medial prefrontal, anterior cingulated, and entorhinal, cortices). Interestingly, DA reuptake is of physiological importance for the elimination of DA in cortical hemispheres, limbic forebrain and striatum, but not in midbrain DA neurons. It is thus possible that DA uptake inhibitors (and amphetamine and modafinil) act mostly on DA terminals of the cortical hemispheres, limbic forebrain, and striatum to induce wakefulness. Local perfusion experiments of DA compounds in rats and canine narcolepsy have suggested that the VTA, but not the SN, is critically involved in EEG arousal regulation (11). DA terminals of the mesolimbocortical DA system may thus be important in mediating wakefulness after DA-related CNS stimulant co-administration.

VI. Clinical Pharmacology of Amphetamine and Amphetamine-Like Compounds

Amphetamine releases not only DA but also NE. NE indirectly stimulates alpha and beta adrenergic receptors, a profile common to all indirectly acting sympathomimetic compounds. This results in significant cardiovascular effects. Alpha-adrenergic stimulation produces vasoconstriction, thereby increasing both systolic and diastolic blood pressure. Heart rate may slow down slightly in reflex (this effect is more pronounced than indirect beta adrenergic stimulation on heart rate at low dose). Smooth muscles respond to amphetamine as they do with other sympathomimetic drugs. There is a

contractile effect on the urinary bladder sphincter-an effect that has been used in treating enuresis and incontinence.

Other acute side effects include mild gastrointestinal disturbance, anorexia, dryness of the mouth, tachycardia, cardiac arrhythmias, insomnia, and restlessness, headaches, palpitations, dizziness, and vasomotor disturbances. Also, agitation, confusion, dysphoria, apprehension, and delirium may occur. Other documented side effects include flushing, pallor, excessive sweating and muscular pains. Tiredness and sleepiness as well as lethargy and listlessness may also occur when the effects wear off, in addition to a mild mood depression. For common side effects of CNS stimulant drugs in narcoleptics, refer to Table 1.

Common side effects occurring during long-term treatment in narcolepsy include irritability, headache, bad temper, and profuse sweating (reported by over one-third of subjects). Other less common side effects include anorexia, gastric discomfort, nausea, talkativeness, insomnia, orofacial dyskinesia, nervousness, palpitations, muscle jerking, chorea, and tremor. Psychiatric symptoms, such as delusions or hallucinations may also occur, but are rather rare in narcoleptic patients that receive amphetamine. Methamphetamine is more frequently associated with central nervous complications because its higher toxicity and central penetrance.

The side-effect profile of methylphenidate is similar to that of amphetamine, but is less sever, and includes nervousness, insomnia, and anorexia, as well as dose-related systemic effects such as increased heart rate and blood pressure. Methylphenidate overdose may lead to seizures, dysrhythmias, or hyperthermia.

Drug-drug interactions with amphetamine and methylphenidate are generally pharmacodynamic/neurochemical in nature. A small portion of the metabolism of amphetamine and methylphenidate occurs via the cytochrome P450 2D6, and drugs that inhibit 2D6 metabolism can theoretically increase plasma levels of amphetamine. This is however, rarely a significant problem with therapeutic doses. Tricyclic drugs inhibit the metabolism of amphetamine and amphetamine-like stimulants and enhance their behavioral effects (12). However in practice, amphetamine, 10–16 mg (and also methylphenidate, 10–60 mg, mazindol, 2–12 mg), have been safely given with imipramine and clomipramine, 10–100 mg to treat narcolepsy-cataplexy (13). The dosage of amphetamine required to control EDS in narcolepsy may be reduced by one-third through the simultaneous use of tricyclic drugs. MAO-A inhibitors (e.g., nialamide, pargyline and tranylcypromi), inhibit the removal of amphetamine by the liver and greatly potentiate the behavioral effects of amphetamine. Coadministration of MAO inhibitors and amphetamine derivatives is generally contraindicated.

VII. Non-Amphetamine Stimulants

A. Modafinil

Modafinil (2-[(diphenylmethyl)sulfinyl]acetamide) is a chemically unique compound developed in France (Figure 1). Modafinil has been available in France since 1984 on a compassionate mode and was approved in France in 1992. Modafinil has recently been approved in the United States for the treatment of narcolepsy, idiopathic hypersomnia, shift-work disorder and for the treatment of residual sleepiness in treated patients with the sleep apnea syndrome. Modafinil is a primary metabolite of adrafinil,

a vigilance-promoting compound discovered in France in 1974. The kinetic study of adrafinil led to the identification of modafinil in 1976. Modafinil lacks the adrafinil's terminal amide hydroxy group (Fig. 1) and is better tolerated.

Modafinil is rapidly absorbed but slowly cleared. It has fairly high protein binding and a Vd of 0.8 L/kg. Its half-life is 11–14 hours. Up to 60% of modafinil is converted into modafinil acid and modafinil sulfone, both of which are inactive metabolites. Metabolism primarily occurs via cytochrome P450 3A4/5, but the compound has also been reported to induce P450 2C 19 in vitro. Modafinil is currently available as a racemic mixture of two active isomers. The elimination profile of the two isomers is reversed in rats versus humans. In humans, the d-isomer is cleared three times faster than l-isomer, and females clear modafinil faster than males. The two isomers may also have slightly different pharmacodynamic properties and the longer acting isomer is currently under development.

Modafinil is one of the few compounds that have been specifically developed for the treatment of narcolepsy. Early trials include a clinical trail by Bastujji and Jouvet (14) in 1988, and the first double blind multicenter trial in 3 French centers and one Canadian center in 1994 (15) have shown that 100–300 mg modafinil is effective in improving daytime sleepiness in narcolepsy and hypersomnia without interfering with nocturnal sleep, but has limited efficacy on cataplexy and other symptoms of abnormal REM sleep. Modafinil is well tolerated and the most frequent reported side effects are, headache, nausea, rhinitis and nervosism (in the descending order). Early clinical trials in France and Canada Pharmacological experiments in canine narcolepsy also demonstrated that modafinil has no effects on cataplexy, while it significantly increases time spent in wakefulness (16). A recent double-blind trial in 18 centers in the United States on 283 narcoleptic subjects revealed that 200 mg and 400 mg of modafinil significantly reduced excessive daytime sleepiness (EDS) and improved patients' overall clinical condition. The study also showed that modafinil was well-tolerated and the most frequent reported side effects were headache and nausea (17).

Several factors make modafinil an attractive alternative to amphetamine-like stimulants. Foremost, animal studies suggest that the compound does not affect blood pressure as much as amphetamines do; only high doses (800 mg) have been found to be associated with higher rates of tachycardia and hypertension. This suggests that modafinil might be useful for patients with a heart condition or high blood pressure. Second, data obtained to date suggest that tolerance and dependence is limited with this compound (14), although a recent animal study suggests cocaine-like discriminative stimulus and reinforcing effects of modafinil in rats and monkeys, respectively. Third, modafinil also has little effects on the neuroendocrine system. A comparison of healthy volunteers who were sleep deprived for 36 hours versus those who received modafinil during sleep deprivation found no difference in cortisol, melatonin, or growth hormone levels. Fourth, clinical experience suggests that the alerting effects of modafinil might be qualitatively different from those observed with amphetamine (14). In general, patients feel less irritable and/or agitated with modafinil than with amphetamines (14) and do not experience severe rebound hypersomnolence once modafinil is eliminated. This differential profile is substantiated by animal experiments. In rats and dogs, modafinil does not increase locomotion beyond the effect expected in association with increased wakefulness (16, 18). Similarly, modafinil acutely decreases both REM and non-REM sleep in rats for up to 5–6 hours, but the effect is not followed by a

rebound hypersomnolence. This profile contrasts with the intense recovery sleep seen following amphetamine-induced wakefulness. Considering the many advantages of modafinil over amphetamine treatment (fewer cardiovascular side effects, lower abuse potential-tolerance, and less rebound sleepiness when the drug effects are waning), modafinil has replaced amphetamine-like stimulants as a first line treatment for EDS.

Current indications for modafinil include narcolepsy and idiopathic hypersomnia. It has also been recently approved by the Food and Drug Administration (FDA) for the treatment of shift work disorder and residual sleepiness in treated sleep apnea patients [generally with continuous positive airway pressure (CPAP)]. There are several reports suggesting that modafinil is also effective for treatment of ADHD, fatigue in multiple sclerosis and EDS in myotonic dystrophy or Prader-Willi Syndrome. Modafinil is also being used in the treatment of recurrent hypersomnia.

The mechanism of action of modafinil is highly debated. An interaction and/or involvement of adrenergic alpha-1 systems was initially suggested by the ability of the alpha-1 antagonist, prazosin, to antagonize modafinil-induced increases in motor activity in mice (19) and wakefulness in cats (20). However, modafinil does not bind to alpha-1 receptors in vivo (Ki $>$10-3M, obtained from prazosin binding using canine cortex) (16). Furthermore, previous studies in the canine model of narcolepsy have shown that adrenergic alpha-1 agonists are potent anticataplectic agents and have a significant acute hypertensive effect. The fact that modafinil has no anticataplectic activity and lacks hypertensive effects rather suggest that its alerting properties are not derived from adrenergic alpha-1 stimulation.

A serotoninergic 5HT2 receptor-mediated change in GABAergic transmission was next suggested (21). Modafinil increases 5HT metabolism in the striatum and reduces GABA flow to the cortex (21). The effect on GABA release is blocked by ketanserin (a 5HT2 antagonist) but not by prazosin (21). Furthermore, muscimol, a GABAergic agonist, blocks the effect of modafinil on wakefulness in cats (20). Although serotonergic/GABAergic interaction may be involved in the mode of action of modafinil, the effects described may be indirect and additional work is needed to substantiate this hypothesis. Modafinil also does not bind serotoninergic receptors in vitro.

In the canine model of narcolepsy, it was observed that selective dopaminergic reuptake inhibitors have no effect on canine cataplexy, but potently promote wakefulness (Fig. 3) (4) (Fig. 3). Modafinil had a similar profile, and it was subsequently found that modafinil has a low but selective affinity for the DAT (4). A lack of wake-promoting effects of modafinil (as well as amphetamine) in DAT knockout mice, clearly demonstrates that an intact DAT molecule is required for mediating modafinil's arousal effect (7) (Fig. 4).

Other investigators have shown, however, that modafinil can be distinguished pharmacologically from most other compounds with presynaptic dopaminergic activity. For example, modafinil does not produce stereotypic behavior at high doses. Additionally, agents that inhibit dopaminergic function such as D1 blockers, D2 blockers and tyrosine hydroxylase blockers, have no effect on modafinil's locomotor-enhancing effects in mice. Finally, an in vitro voltametry study found that modafinil did not increase the cathecol oxidation peak height (and indirect measure of dopaminergic activity), suggesting a lack of presynaptic dopaminergic involvement of modafinil activity. Ferraro et al (22), however, reported that systemic administration of modafinil

(30–300 mg/kg) dose-dependently increased DA release in the nucleus accumbens in rats, but these authors claimed that the DA-releasing action of modafinil was most likely secondary to its ability to reduce local GABAergic transmission.

Not only is the exact molecular target of modafinil action uncertain but there is much debate regarding modafinil's neuroanatomical site of action. Anatomical studies coupled with functional markers of neuronal activity (i.e., the immediate early gene product, Fos) have been used to determine activation patterns induced by modafinil in comparison with other stimulants (23). In cats, amphetamine and methylphenidate induce c-Fos throughout the cortex, striatum, and other brain regions. In contrast, modafinil induces a much more restricted pattern of neuronal activation, with marked expression of c-Fos in neurons of the anterior hypothalamus area and suprachiasmatic nuclei-brain regions that have been implicated in sleep and circadian regulation (23). Modafinil also increases c-Fos expression in hypocretin cells (24,25) and histaminergic cells of the tuberomammilary nucleus; these effects have been suggested to mediate the wake promoting effects of modafinil. At higher doses, the striatum and cingulate cortex are also activated (25). Of note, however, it is likely that the stimulation of hypocretin cells is not essential to induce wakefulness since both hypocretin receptor-2 mutated canine narcolepsy and hypocretin-ligand deficient human narcolepsy (90% of narcolepsy-cataplexy patients) respond well to modafinil treatment. More likely, activation of these cells groups is secondary to the expression of increased wakefulness, as c-Fos expression in these cell groups increases in naturally occurring wakefulness.

Gallopin et al, recently reported that modafinil inhibits the sleep-active neurons of the ventrolateral preoptic nucleus (VLPO, a sleep promoting network of neurons), by facilitating adrenergic neurotransmission (26). In this study, modafinil potentiated the inhibitory effects of norepinephrine on VLPO neurons in a slice preparation. Surprisingly, modafinil did not potentiate the inhibitory effects of dopamine or serotonin on VLPO neurons. Nisoxetine, a potent NET inhibitor with low affinity to DAT (4), had a similar effect and the response to the two drugs was not additive, suggesting they might work through the same biochemical pathways. Since modafinil does not bind to the NET (4) and NE uptake inhibitors do not possess strong wake-promoting effects, modafinil may modulate norepinephrine/dopamine uptake mechanisms through novel mechanisms. In this case, modafinil may work on both the DA and NE system to promote wakefulness, and adrenergic/DAT interactions may be involved. Of note however, very high modafinil concentrations (generally 200 μM, the maximum that can be dissolved) were used in this study in vitro. It may also be that at this very high dose, small effects on adrenergic uptake, undetectable with the usual radio receptor binding assays, could occur.

Further studies of action of modafinil will be needed to clarify the above discrepancies regarding its modes of action and may lead to new and interesting insights regarding the mode of action of stimulant medications in general.

B. Mazindol

Mazindol (2–8 mg daily), a sympathomimetic anorexisant, is rarely used in the United States. At these doses, mazindol produces central stimulation, a reduction in appetite and an increase in alertness, but has little or no effect on mood or the cardiovascular system. Mazindol is a weak releasing agent for DA that also blocks both DA and NE

reuptake. Mazindol has a high affinity for the DA and NE transporters (4), yet interestingly this compound has a low abuse potential. It is effective for the treatment of both EDS and cataplexy in humans (27) and in canine narcolepsy (28), possibly due to its dual dopaminergic and noradrenergic effects (4). Narcoleptics are relatively well tolerated to mazinzol, and reported side effects are insomnia, headache, anorexia, constipation, chest discomfort, delayed ministration, but drug tolerance may occur in some patients. However, higher doses of mazindol reported to cause other side effects including gastrointestinal discomfort, nervousness, dry mouth, nausea, urinary retention, vomiting, tremor and angioneurotic edema. Therefore, mazindol may not be recommended for treatment of severe narcoleptic subjects that require large doses.

C. Bupropion

Bupropion is a DA reuptake inhibitor that may be useful for the treatment of EDS associated with narcolepsy (100 mg t.i.d.) (4,29). It may be especially useful in cases associated with atypical depression (29). Bupropion was synthesized in 1966 by a group seeking new antidepressants chemically related to tricyclic antidepressants, but without any significant sympathomimetic, cholinolytic, or monoamine oxidase inhibitory properties. Bupropion is classified as a monocyclic phenylbuthylamine of the aminoketone group. It selectively blocks DA uptake, and is 6 times more potent than imipramine, and 19 times more potent than amitriptyline in blocking DA reuptake. The selectivity of bupropion for the dopamine transporter is not absolute. Bupropion is a weak competitive inhibitor of NE reuptake (65-fold less potent than imipramine). Very limited serotonergic effects are also observed (200-fold less potent than imipramine). Bupritropion is generally well-tolerated, and most commonly reported side effects include headache, nausea, drymouth and insomnia. Convulsion is a dose dependent risk of bupropion (0.1% at 100–300 mg, and 0.4% at 400 mg).

D. Selegiline (L-Desprenyl)

Selegiline is a methamphetamine derivative and a potent, irreversible, MAO-B selective inhibitor primarily used for the treatment of Parkinson's disease. Low doses of selegiline do not necessitate dietary restriction as usually required with other irreversible MAOIs. Ten mg of selegiline daily has no effect on the symptoms of narcolepsy, but 20–30 mg improves alertness and mood, and reduces cataplexy, an effect comparable to d-amphetamine at the same dose (30). Selegiline may be an interesting alternative to the use of more classical stimulants, as its potential for abuse has been reported to be very low.

The fact that this compound is often considered as a simple MAO-B inhibitor rather than an amphetamine precursor deserves special mention. Selegiline does not only inhibit MAO-B irreversibly, it also metabolizes into amphetamine (20–60% in urine) and methamphetamine (9–30% in urine). In the canine model of narcolepsy, selegiline (2 mg/kg p.o) was demonstrated to be an effective anticataplectic agent, but this effect was found to be mediated by its amphetamine metabolites rather than via MAO-B inhibition (31). Several trials in human narcolepsy have demonstrated a good therapeutic efficacy of selegiline on both sleepiness and cataplexy with relatively few side effects (30, 32), but this efficacy is also likely to partially mediated by amphetamine metabolites.

E. Caffeine

Caffeine may be the most popular and widely consumed CNS-stimulant in the world. An average cup of coffee contains 50 to 150 mg of caffeine. Tea, cola drinks, chocolate, and cocoa all contain significant amounts of caffeine. Caffeine can also be bought over the counter (OTC) (No Doz®, 100 mg caffeine; Vivarin® 200 mg caffeine), and is commonly used by narcoleptic patients prior to diagnosis.

Taken orally, caffeine is rapidly absorbed. The half-life of caffeine is 3.5–5 hours. The behavioral effects of caffeine include increased mental alertness, a faster and clearer flow of thought, wakefulness, and restlessness. Fatigue is reduced and sleep-onset delayed (33). The physical effects of caffeine include palpitations, hypertension, increased gastric acid secretion and increased urine output (33). Heavy consumption (12 or more cups a day, or 1.5 g of caffeine) causes agitation, anxiety, tremors, rapid breading and insomnia.

Caffeine is a xanthine derivative. The mechanism of action of caffeine on wakefulness involves non-specific adenosine receptor antagonism. Adenosine is an endogenous sleep-promoting substance with neuronal inhibitory effects. In animals, sleep can be induced after administration of metabolically stable adenosine analogues with adenosine A1 receptors (A1R) or A2A receptors (A2AR) agonistic properties, such as N6-L-(phenylisopropyl)-adenosine, adenosine-5′-N-ethylcarboxamide, and cyclohexyladenosine (34). Adenosine content is increased in the basal forebrain after sleep deprivation. Adenosine has thus been proposed to be a sleep-inducing substance accumulating in the brain during prolonged wakefulness (35).

Most studies in the area of sleep and adenosinergic effects have focused on A1R-mediated effects. The rationale for this focus is that A1R are widely distributed in the CNS, whereas A2AR are discretely localized in the striatum, nucleus accumbens and olfactory bulb. Interestingly, sleep wake patterns and response to sleep deprivation were recently examined in adenosine receptor A1R knockout mice and found to be generally unaltered, suggesting that the constitutional lack of adenosine A1R does not prevent homeostatic regulation of sleep. In contrast, the sleep inhibitory effect of 8-cyclopentyltheophylline (a selective A1R antagonist) was abolished in these animals, indicating A1R mediation of stimulant effects with this compound.

VIII. Future Stimulant Treatments

It is now revealed that the sleep disorder narcolepsy is caused by impairments of hypocretin (orexin) transmission in animal models and humans (36). Most notably, human narcolepsy is caused by a dramatic decrease in hypocretin levels in the brain and the CSF. This raises the possibility that hypocretin-based stimulant therapies may be designed in the future. Hypocretins-1 and -2 are produced by a small group of neurons localized in the lateral hypothalamus. The neurons project to the olfactory bulb, cerebral cortex, thalamus, hypothalamus and brainstem, particularly the locus coeruleus (LC), VTA, raphe nucleus and to cholinergic nuclei and cholinoceptive site (such as the pontine reticular formation), thought to be important for the sleep regulation.

A series of studies have shown that the hypocretin system is a major excitatory system that controls the activity of monoaminergic and cholinergic systems with major effects on vigilance states (36). Hypocretins potently excite most monoaminergic neurons, adrenergic LC, dopaminergic VTA, serotonergic raphe and histaminergic

tuberomammillary neurons (36). It is thus likely that a deficiency in hypocretin neurotransmission induces an imbalance between these classical neurotransmitter systems, with primary effects on sleep-state organization and vigilance.

The potential effect of hypocretin injections on wakefulness has been explored by several groups. Whereas the effect of peripherally administered hypocretins is controversial (37,38), central administration of hypocretin-1 (more stable than hypocretin 2) strongly promotes wakefulness in animals (36,37). Recent experiments in canine narcolepsy (receptor-mutated and ligand deficient) suggest that stable and centrally active hypocretin analogs (possibly non-peptide synthetic hypocretin ligands) will need to be developed in order to be effective peripherally (37). This is also substantiated by a recent study that found normalization of sleep/wake patterns and behavioral arrest episodes (equivalent to cataplexy and REM sleep onset) in orexin/hypocretin deficient mice knockout models supplemented by central administration of hypocretin-1 (39).

Another potential for drug development in the area of wake-promotion are histaminergic compounds. Histamine has long been implicated in the control of vigilance, and H1 antagonists are strongly sedative. The downstream effects of hypocretins on the histaminergic system (hcrtr2 excitatory effects) are likely to be important in mediating the wake-promoting properties of hypocretin (40). In fact, brain histamine and CSF histamine contents are reduced in narcoleptic subjects (41,42). Although centrally injected histamine or histaminergic H1 agonists promote wakefulness, systemic administration of these compounds induces various unacceptable side effects via peripheral H1 receptor stimulation. In contrast, the histaminergic H3 receptors are regarded as inhibitory autoreceptors, and are enriched in the central nervous system. H3 antagonists enhance wakefulness in normal rats and cats (43) and in narcoleptic mice models (44). Histaminergic H3 antagonists might be a useful as wake-promoting compounds for the treatment of EDS or as cognitive enhancers and are under study in several pharmaceutical companies.

Other pathways with possible applications in the development of novel stimulant medications include the adenosinergic system (see above), the adrenergic system (for example some NE-reuptake inhibitors), the GABAergic system (for example, inverse benzodiazepine agonists), the glutamatergic system (ampakines) and TRH analogues (3).

IX. Conclusion

Amphetamine-like stimulants have been used in the treatment of narcolepsy and various other conditions for decades, yet only recently has the mode of action of these drugs on vigilance been characterized. In almost all cases, the effects on vigilance were found to be mediated via effects on the DA transporter, DAT. This has generally led to the widely accepted hypothesis that wake-promoting effects will be impossible to differentiate from abuse potential effects for these compounds. Importantly however, the various mediations available have differential effects and potency on the DA transporter and on monoamine storage/release. The various available stimulants are more or less selective for dopamine versus other amines. Even if much work remains to be done in this area, it appears more and more likely that complex properties (for example, the ability to release DA rather than simply block reuptake, plus the combined effects on other monoamines [such as serotonin]) may be important to explain abuse potential. Differential binding properties on the DAT transporter itself may also be involved, together with drug potency and compound solubility. The lack of solubility of some low potency

compounds may for example, result in an inability to administer the drug via the nasal passage or intravenously. Finally, lower abuse potential for these compounds has long been suspected in narcolepsy-cataplexy patients, either because of the biochemical hypocretin abnormality or because of the social aspects of treating narcolepsy as a disease.

The mode of action of the modafinil remains controversial and may involve dopaminergic and/or non-dopaminergic effects. Whatever its mode of action is, the compound is generally found to be safer and to have a lower abuse potential than amphetamine stimulants. Its favorable side effects profile has led to an increasing use outside the narcolepsy indication, most recently in the context of shift work disorder and residual sleepiness in treated sleep apnea patients. This recent success exemplifies the need for developing novel wake-promoting compounds with low abuse potential. A need for treating daytime sleepiness extends well beyond the relatively rare indication of narcolepsy-cataplexy.

References

1. Hartmann A, Cravens J. Sleep: effect of d- and l-amphetamine in man and rat. Psychopharmacology 1976; 50:171–175.
2. Kuczenski R, Segal DS. Neurochemistry of amphetamine. In: Cho AK, Segel DS, eds. Psychopharmacology, toxicology and abuse. San Diego: Acdemic Press, 1994:81–113.
3. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol 1997; 52:27–78.
4. Nishino S, Mao J, Sampathkumaran R, et al. Increased dopaminergic transmission mediates the wake-promoting effects of CNS stimulants. Sleep Research Online 1998; 1:49–61. http://www.sro.org/1998/Nishino/49/.
5. Kanbayashi T, Nishino S, Honda K, et al. Differential effects of D-and L-amphetamine isomers on dopaminergic trasmission: Implication for the control of alertness in canine narcolepsy. Sleep Res 1997; 26:383.
6. Kuczenski R, Segal DS, Cho A, et al. Hippocampus norepinephrine, caudate dopamine and serotonin and behavioral responses to the stereoisomers of amphetamine and methamphetamine. J Neurosci 1995; 15:1308–1317.
7. Wisor JP, Nishino S, Sora I, et al. Dopaminergic role in stimulant-induced wakefulness. J Neurosci 2001; 21:1787–1794.
8. Xu F, Gainetdinov RR, Wetsel WC, et al. Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. Nat Neurosci 2000; 3:465–471.
9. Takahashi N, Miner LL, Sora I, et al. VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci U S A 1997; 94:9938–9943.
10. Björklund A, Lindvall O. Dopamine-containing systems in the CNS. In: Björklund A, Hökfelt T, eds. Handbook of Chemical Neuroanatomy, Vol 2, Classical Transmitter in the CNS, Part I. Amsterdam: Elsevier, 1984:55–121.
11. Honda K, Riehl J, Mignot E, et al. Dopamine D3 agonists into the substantia nigra aggravate cataplexy but do not modify sleep. NeuroReport 1999; 10:3717–3724.
12. Cooper TB, Simpson GM. Concomitant imipramine and methylphenidate administration: a case report. Am. J. Psychiatry 1973; 130:6.
13. Zarcone V. Narcolepsy: A review of the syndrome. N Engl J Med 1973; 288:1156–1165.
14. Bastuji H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog. Neuropsychopharmacol. Biol. Psychiat. 1988; 12:695–700.
15. Billiard M, Besset A, Montplaisir J, et al. Modafinil: A double-bling multicentric study. Sleep 1994; 17:S107–S112.
16. Shelton J, Nishino S, Vaught J, et al. Comparative effects of modafinil and amphetamine on daytime sleepiness and cataplexy of narcoleptic dogs. Sleep 1995; 18:817–826.
17. Fry J, group TMs. A new alternative in the pharmacologic management of somnolence: A phase III study of modafinil in narcolepsy, Am Neurol Assoc, Miami, FL, 1996.

18. Edgar DM, Seidel WF, Contreras P, et al. Modafinil promotes EEG wake without intensifying motor activity in the rat. Canadian Journal Physiology and Pharmacology 1994; 72, S1:362.
19. Duteil J, Rambert FA, Pessonnier J, et al. Central a1-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil: studies with experimental animals. Eur J Pharmacol 1990; 180:49–58.
20. Lin JS, Roussel B, Akaoka H, et al. Role of catecholamines in modafinil and amphetamine induced wakefulness, a comparative pharmacological study. Brain Res 1992; 591:319–326.
21. Tanganelli S, Fuxe K, Ferraro L, et al. Inhibitory effects of the psychoactive drug modafinil on gamma-aminobutyric acid outflow from the cerebral cortex of the awake freely moving guinea pig. Arch Pharmacol 1992; 345:461–465.
22. Ferraro L, Tananelli S, O'Connor WT, et al. The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. Eur J Pharmacol 1996; 306:33–39.
23. Lin JS, Hou Y, Jouvet M. Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-*fos* immunocytochemistry in the cats. Proc Natl Acad Sci USA 1996; 93:14128–14133.
24. Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999; 98:437–451.
25. Scammell TE, Estabrooke IV, McCarthy MT, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000; 20:8620–8628.
26. Gallopin T, Luppi PH, Rambert FA, et al. Effect of the wake-promoting agent modafinil on sleep-promoting neurons from the ventrolateral preoptic nuclueus: an In vitro phramacologic study. Sleep 2004; 27:19–25.
27. Iijima S, Sugita Y, Teshima Y, et al. Therapeutic effects of mazindol on narcolepsy. Sleep 1986; 9:265–268.
28. Nishino S, Mao J, Sampathkumaran R, et al. Adrenergic, but not dopaminergic, uptake inhibition reduces REM sleep and cataplexy concomitantly. Sleep Res 1997; 26:445.
29. Rye DB, Dihenia B, Bliwise DL. Reversal of atypical depression, sleepiness, and REM-sleep propensity in narcolepsy with bupropion. Depress Anxiety 1998; 7:92–95.
30. Hublin C, Partinen M, Heinonen EH, et al. Selegiline in the treatment of narcolepsy. Neurology 1994; 44:2095–2101.
31. Nishino S, Arrigoni J, Kanbayashi T, et al. Comparative effects of MAO-A and MAO-B selective inhibitors on canine cataplexy. Sleep Res 1996; 25:315.
32. Roselaar SE, Langdon N, Lock CB, et al. Selegiline in narcolepsy. Sleep 1987; 10:491–495.
33. Rall TR. Central nervous system stimulants. In: Gilman AG, Goodman LS, Rall TW, et al., eds. The Pharmacological Basis of Therapeutics, 7th Ed. New York: Pergamon Press, 1985:345–382.
34. Radulovacki M, Virus RM, Djuricic-Nedelson M, et al. Adenosine analogs and sleep in rats. J Pharmacol Exp Ther 1984; 228:268–274.
35. Porkka-Heiskanen T, Strecker RE, Thakkar M, et al. Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science 1997; 276:1265–1268.
36. Willie JT, Chemelli RM, Sinton CM, et al. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 2001; 24:429–458.
37. Fujiki N, Ripley B, Yoshida Y, et al. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin ligand deficient narcoleptic dog. Sleep 2003; 6:953–959.
38. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Research Online 2000; 3:23–28.
39. Mieda M, Willie JT, Hara J, et al. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci U S A 2004.
40. Huang ZL, Qu WM, Li WD, et al. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci U S A 2001; 98:9965–9970.
41. Nishino S, Fujiki N, Ripley B, et al. Decreased brain histamine contents in hypocretin/orexin receptor-2 mutated narcoleptic dogs. Neurosci Lett 2001; 313:125–128.
42. Nishino S, Sakurai E, Nevisimalova S, et al. CSF histamine content is decreased in hypocretin-deficient human narcolepsy. Sleep 2002; 25 (suppl):A476.
43. Lin JS, Sakai K, Vanni-Mercier G, et al. Involvement of histaminergic neurons in arousal mechanisms demonstrated with H3-receptor ligands in the cat. Brain Res 1990; 523:325–330.
44. Shiba T, Fujiki N, Wisor J, et al. Wake promoting effects of thioperamide, a histamine H3 antagonist in orexin/ataxin-3 narcoleptic mice. Sleep 2004; (suppl):A241–A242.

49
Anticataplectic Medications

SEIJI NISHINO
Stanford University School of Medicine, Stanford, California, U.S.A.

I. Introduction

Cataplexy, is a sudden drop of antigravity-muscle tone triggered most often by positive emotional factors (such as laughter, winning a game, responding with a witty remark) (1). Cataplexy is pathognomonic for narcolepsy, and it occurs almost exclusively in idiopathic narcolepsy and in rare cases of symptomatic narcolepsy (i.e., narcolepsy associated with other neurological conditions such as a brain tumor or stroke). Approximately sixty percent of narcoleptic subjects diagnosed by the current International Classification of Sleep Disorders (ICSD) Diagnostic Criteria develop cataplexy.

Cataplexy is currently treated pharmacologically, most often with tricyclic antidepressants (1). The anticataplectic effect of tricyclic antidepressant (imipramine) was coincidentally found in the clinical practices of the 1950s. Tricyclic antidepressants are also effective for reducing sleep paralysis and hypnagogic hallucinations, but have little effect on excessive daytime sleepiness (1). Conversely, amphetamines (and amphetamine-like compounds) and modafinil are used for treatment of excessive sleepiness, but have little or no anticataplectic effects. Monoamine oxidase inhibitors (MAOIs) have also occasionally been used as anticataplectics (1). Most recently, anticataplectic effects of gamma hydroxybutyrate (GHB, a short-lasting hypnotic given at night) has also been recognized (1).

Occurrence of cataplexy is tightly associated with deficiency in hypocretin neurotransmission (the ligand deficiency for human, mice, and dogs, and the receptor 2 mutation for dogs and mice) (2,3). Although hypocretin replacement therapies are reported to be effective for cataplexy in animal models, this therapeutic option in humans is not yet available, mostly due to the lack of centrally penetrable hypocretin agonists.

In this chapter, pharmacological and neurochemical aspects of various anticataplectics are discussed.

II. Pharmacology of Antidepressants

A. Historical View

Antidepressants are drugs that are effective for treating depression and are represented by a diverse group of chemical structures. Many hypotheses about how these

antidepressants work exist and, usually, these theories involve biogenic amine neurotransmitters, especially norepinephrine (NE), and serotonin (5-HT) (4).

Of the many antidepressants, one of the first types discovered and, until about 10 years ago, the most widely prescribed are the tricyclics, so named because of the three fused rings in the structure. The prototype of this class of compound, imipramine, was initially synthesized for use as an antihistamine (H1) but was discovered to have antidepressant properties as the result of astute clinical observations on depressed schizophrenic patients (4). Imipramine was selected for a trial in schizophrenia because of its close structural similarity to chlorpromazine. This finding led to the study of imipramine in clinical trials in depressed patients, which subsequently proved its efficacy as an antidepressant (4).

Another class of antidepressants, MAOI, were introduced in the 1950s because of observations both in the clinic and in the laboratory (5). The first compound of this group to be tested and proven effective as an antidepressant was iproniazid, an antituberculous drug. This research began after scientists observed that iproniazid caused euphoria and elation in some patients treated for tuberculosis and that it reversed the apparent sedation caused by the drug reserpine in laboratory animals. MAOIs are used much less frequently than other types of antidepressants because of the "cheese reaction," a marked and potentially fatal increase in blood pressure that can occur when a patient being treated with one of these drugs ingests foods with high tyramine content (such as aged cheese, bananas, smoked and pickled fish, fermented sausages) (5). The excess tyramine, which cannot be degraded by monoamine oxidase, displaces neuronal stores of catecholamines (e.g., norepinephrine [NE]) that ultimately increase blood pressure. Consequently, patients taking MAOIs are required to be on a tyramine-free diet and must also avoid the use of sympathomimetic stimulants.

B. Mechanisms of Action

Shortly after the discovery of tricyclic antidepressants, it was shown that these drugs acutely blocked reuptake by the nerve ending of both NE and 5-HT (4). In general, tertiary amine tricyclic antidepressants, such as imipramine or clomiplamne were potent blockers of 5-HT reuptake but weak blockers of NE transport, whereas the converse seemed true for the secondary amine compounds (6) (see also Table 2 below). It was also shown that MAOIs increase brain levels of catecholamines and serotonin by preventing their degradation (5). Thus, it was thought that the therapeutic effect of the antidepressant was due to increased duration of elevated levels of monoamines in the synaptic cleft (i.e., monoamine hypothesis), but the exact mechanisms of the action of antidepressants on depressive symptoms are not known (4). One of the complex issues is the fact that the mood-elevating effect of antidepressants does not become apparent until at least 7 to 10 days to several weeks after treatment has started; longer-term mechanisms need to be considered. This sharply contrasts to an immediate occurrence of anticataplectic effects by these compounds when they are used in narcoleptic patients (1).

Most of classical tricyclics also have the ability to block several neurotransmitter receptors, such as at histaminergic H1, muscarinic acetylcholinergic, alpha1-adrenergic, and serotonergic 5-HT2A receptors (4). These pharmacological effects occur immediately and are probably not related to its major mode of action but underlie various

adverse effects. For example, muscarinic receptor blockade can cause dry mouth and constipation. Histamine H1 receptor antagonism can cause sedation and drowsiness. Some antidepressants (such as tricyclic antidepressant doxepin) have such high affinity for the H1 receptor that they are among the most potent histamine H1 antagonists available. Thus, doxepin is also used for the treatment of allergy and certain dermatological problems.

The newer second-generation antidepressants usually are more selective at blocking the reuptake of 5-HT over NE. This has led to the concept of the serotonin-selective reuptake inhibitor or SSRI (7). The first of this group and the most widely prescribed, until recently, was fluoxetine, which was first marketed in the United States in 1988. Since then, several others of this class have been marketed. These include sertraline, paroxetine, and citalopram. Another drug, venlafaxine, is marketed as a 5-HT and NE reuptake inhibitor (SNRI) antidepressant. Another SNRI milnacipran is available in some European countries and Japan. A selective NE blocker atomoxetin was also introduced in the US market and used for the treatment of attention deficit hyperactive disorder. Another selective NE blocker, reboxetine, is only available in some European countries.

Because the newer second-generation drugs usually have lower affinities for the above-mentioned receptors, they are less likely to cause the adverse effects commonly seen with the older antidepressants.

III. Canine Narcolepsy Model for Understanding the Pharmacological Control of Cataplexy

Canine narcolepsy is a naturally occurring animal model of the human disease, and both sporadic and familial narcoleptic cases exist in canines (1). Beside the discovery of canine narcolepsy gene by a positional cloning project (see canine narcolepsy section), neuropharmacological and neurochemical understandings of narcolepsy (i.e., wakefulness and cataplexy) also have been greatly facilitated using these canine models (1). Since cataplexy can be easily elicited by food or play and the severity of cataplexy can be quantified by a simple behavioral assay (i.e., Food Elicited Cataplexy Test), these animals have been intensively used for evaluating anticataplectic effects of various compounds (1). Similarly narcoleptic dogs are also very useful to study physiological and pathophysiological aspects of cataplexy and REM sleep atonia (8).

A. REM Sleep vs. Cataplexy: Physiology and Pathophysiology

After the discovery of sleep onset REM periods (SOREMPs) in narcoleptic patients, narcolepsy has often been regarded as a disorder of REM sleep generation. REM sleep usually appears 90 minutes after sleep onset and reappears every 90 minutes in humans (30 minutes in dogs). Therefore, it was thought that in narcolepsy, REM sleep phenomenology could intrude in active wake or at sleep onset, resulting in cataplexy, sleep paralysis and hypnagogic hallucinations. These 3 symptoms are often categorized as "dissociated manifestations of REM sleep" (1). During REM sleep, complete and "tonic" inhibition of muscle tonus together with "phasic" bursts

of rapid eye movements (REMs), occurs physiologically, and the amplitude of electromyogram is very low (9). However, the activity of pyramidal tract neurons, in the motor cortex that mediate the limb movements, are as high during REM sleep as during active wake. It is thought that the tonic inhibitory signal for muscle tonus originating in the dorsal pons overcomes this pyramidal motor activation system during REM sleep, and this results in complete muscle atonia during this sleep state. This notion is supported by brain lesion studies in animals: when the lesions are made in dorsal pons, the phenomenon called "REM sleep without atonia" is observed, and the animal moves their limbs during REM sleep (9). Abnormal generation of REM sleep might therefore be central to narcolepsy, but this has not been previously demonstrated experimentally.

We have, therefore, analyzed the REM sleep and cataplexy cyclicity in narcoleptic and control canines to observe whether the cyclicity at which REM sleep occurs is disturbed in narcoleptic canines (8). Interval histograms for REM sleep episodes revealed that a clear 30-minute cyclicity exists in both narcoleptic and control animals, suggesting that the system controlling REM sleep generation is intact in narcoleptic dogs (Fig. 1). In contrast to REM sleep, cataplexy can be elicited anytime upon emotional stimulation (i.e., no 30-minute cyclicity is observed) (8). More interestingly, we found that stimulation of a cholinoceptive site (by carbachol or physostigmine injection) in the basal forebrain induces cataplexy-like atonia in narcoleptic dogs, but it induces wakefulness in control dogs. Detailed analysis of this phenomenon further indicate that bursts of rapid eye movements (a phasic phenomenon of REM sleep) in 30 minute cycles that can be observed at the baseline still preserved during carbachol-induced long episodes of cataplexy (10). These results suggest that sites and mechanisms responsible for occurrence of cataplexy and REM sleep may be different, and more global brain structures may possibly be involved in the induction of cataplexy.

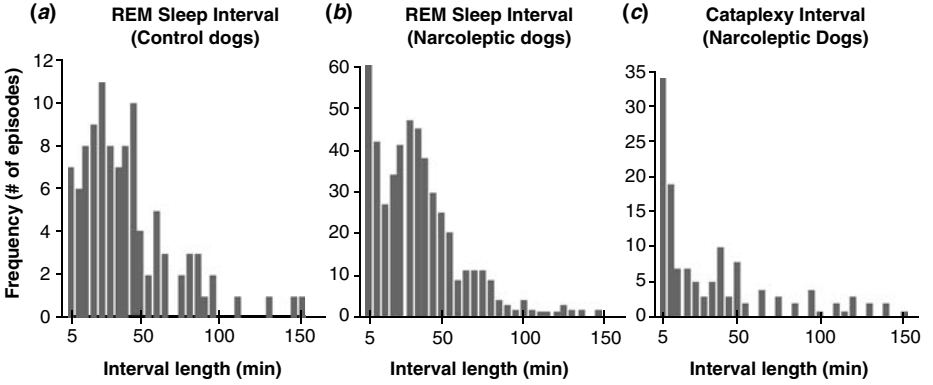


Figure 1 Frequency of interval lengths between consecutive REM sleep episodes in narcoleptic and control dogs and cataplexy interval lengths in narcoleptic canines. REM sleep intervals are shown in 5-minute bins, while cataplexy intervals are shown in 2.5-minute bins. (***a,b***) A clear 30-minute interval between consecutive REM sleep episodes is present in both narcoleptic and control animals. (***c***) No such cyclicity is present for cataplexy.

These results taken together with the results of recent human studies show that cataplexy is tightly associated with hypocretin deficient status, suggesting that cataplexy appears now to be a unique pathological condition caused by a loss of hypocretin neurotransmission (3). In contrast, other REM sleep abnormalities, such as sleep onset REM periods, sleep paralysis, and hypnagogic hallucinations are often seen in sleep disorders other than narcolepsy-cataplexy (11). It should be also noted that cataplexy cannot be induced in these concisions even under special circumstances, such as after selective REM sleep deprivation. These findings also support the hypothesis that the mechanisms for the triggering of cataplexy and REM sleep are distinct.

However, various similarities between REM sleep atonia and cataplexy are also evident. Since H-reflex activity (one of the monosynaptic spinal electrically-induced reflexes) profoundly diminishes or disappears during both REM sleep and cataplexy, it is likely that the motor inhibitory components of REM sleep are also responsible for the atonia during cataplexy (12). Thus, the executive systems for the induction of muscle atonia during cataplexy and REM sleep are likely to be the same. This interpretation is also supported by the pharmacological findings that most compounds that significantly reduce or enhance REM sleep, reduce and enhance cataplexy respectively. However, some exceptions such as discrepant effects of D2/D3 antagonists on REM sleep and cataplexy also exist (13) (see below).

B. Monoaminergic and Cholinergic Interactions in the Control of Cataplexy

The importance of increased cholinergic activity in triggering REM sleep or REM sleep atonia is well established (9). Similarly, activation of the cholinergic systems using physostigmine (cholinesterase inhibitor) also greatly exacerbates cataplexy in canine narcolepsy, but with various side-effects (1). This cholinergic effect is mediated via muscarinic receptors since muscarinic stimulation aggravates cataplexy, while its blockade suppresses it, and nicotinic stimulation or blockade has no effect (1). Application of muscarinic antagonists in human narcolepsy is, however, hampered due to its peripheral side effects.

Monoaminergic transmission is also critical for the control of cataplexy. All therapeutic agents currently used to treat cataplexy (i.e., antidepressants or monoamine MAOIs), are known to act on these systems. Furthermore, whereas a subset of cholinergic neurons are activated during REM sleep, the firing rate of monoaminergic neurons in the brainstem (such as in the locus coeruleus (LC) and the raphe magnus) are well known to be dramatically depressed during this sleep stage (14). In contrast, dopaminergic neurons in the ventral tegmental area (VTA) and substantia nigra (SN) do not significantly change their activity during natural sleep cycles (14). Using canine narcolepsy, it was recently demonstrated that adrenergic LC activity is also reduced during cataplexy (15).

C. Preferential Involvement of Adrenergic Neurotransmission in the Control of Canine Cataplexy

As mentioned above, tricyclic antidepressants have a complex pharmacological profile that includes monoamine reuptake inhibition, anticholinergic, alpha-1 adrenergic

antagonistic and antihistaminergic effects, making it difficult to conclude which one of these pharmacological properties is actually involved in their anticataplectic effects.

In order to determine which property was most relevant, we studied the effects of a large number (a total of 17 compounds) of reuptake blockers/release enhancers specific for the adrenergic, serotonergic or dopaminergic systems. Adrenergic reuptake inhibition was found to be the key property involved in the anticataplectic effect (Table 1) (16). Serotonergic reuptake blockers were only marginally effective at high doses and the dopaminergic reuptake blockers were completely ineffective. Interestingly, it was later found that these DA reuptake inhibitors had potent alerting effects in canine narcolepsy (1).

We also compared the effects of several antidepressants with those of their demethylated metabolites. Many antidepressants (most typically tertiary amine tricyclics) are known to be hepatically first-pass metabolized into their demethylated metabolites that have longer half-lives and higher affinities for adrenergic reuptake sites (17). During chronic drug administration, these demethylated metabolites accumulate (17) and can thus be involved in the drug's therapeutic action. The effects of five available antidepressants (amitriptyline, imipramine, clomipramine, zimelidine, and fluoxetine) were compared with those of their respective demethylated metabolites (nortriptyline,

Table 1 Effect of Selective Monoamine Uptake Inhibitors and Release Enhancers on Canine Cataplexy

Drugs	Dose range (μg/kg i.v.)	Effect on cataplexy	ED 50 (μg)
NE selective compounds			
1. *NE uptake blockers*			
Desipramine	2–500	0[1] ($p = 0.002$)	20
Nisoxetine	1.58–1000	($p = 0.004$)	18
Nortriptyline	2–500	0 ($p = 0.004$)	28
Tomoxetine	16–1000	0 ($p = 0.002$)	11
Viloxazine	8–2000	0 ($p = 0.002$)	128
2. *NE release enhancers*			
Amphetamine[a]	2–128	0 ($p = 0.02$)	37
5-HT selective compounds			
1. *5-HT uptake blockers*			
Fluoxetine	62–4000 $\pm$[2] ($p = 0.06$)		
Indalpine	62–4000 $=$[3] (ns)		
Zimelidine	62–4000 = (ns)		
2. *5-HT release enhancers*			
Fenfluramine	62–4000 $\pm$ ($p = 0.05$)		
DA selective compounds			
1. *DA uptake blockers*			
Amineptine	62–4000 = (ns)		
Bupropion	16–1000 = (ns)		
GBR 12909	16–1000 = (ns)		
2. *DA release enhancers*			
Amfonelic acid	2–128 = (ns)		

[a]Also a dopamine and serotonin release enhancer. [1]: 0 means that for higher doses there was a total suppression of cataplexy in all dogs. [2]: $\pm$ means that there was a decreasing trend in cataplexy during the test, but without total suppression at the higher doses. [3]: $=$ means that there was no change during the test.

Table 2 Anticataplectic Potency of Various Uptake Inhibitors in Respect to In Vitro Potency for Noradrenergic and Serotonergic Uptake Inhibition

			Effect on cataplexy		IC50 (nM)	
Compounds	Dose-range (μg/kg i.v.)	N	ANOVA (p value)	ED50 (μg/kg i.v.)	NE	5-HT
Amitriptyline	2.0–500	6	0.003	123.4	130	300
Nortriptyline	2.0–500	6	0.0004	54.3	30	1400
Imipramine	8.0–500	6	0.01	116.8	60	490
Desipramine	8.0–500	6	0.0005	18.2	2	2000
Zimelidine	8.0–2000	6	(0.08)	(1398.0)	8200	330
Norzimelizine	8.0–2000	6	0.0004	411.7	560	56
Fluoxetine	15.6–250	6	0.04	346.1	4500	170
Norfluoxetine	15.6–250	6	0.01	77.8	10000	220

ED50s were calculated by the equation [Effect 1/4 Maximal Effect/(1 + (ED50/dose))] from the respective dose-response curve of each compound. Data for in vitro inhibition of uptake of NE and 5HT are from Fuller et al. (1977), Ross (1979), and Sulser and Mobley (1980), where the in vitro uptake inhibition of radio-labeled NE and 5-HT into sypaptosomes was measured using rat forebrain tissue. The correlation between in vivo anticataplectic effects and in vitro serotonergic uptake inhibition was weak and negative while a strong positive correlation between in vivo anticataplectic effects and in vitro adrenergic uptake inhibition was observed among the 10 compounds tested.

desipramine, desmethylclomipramine, norzimelidine and norfluoxetine) (Table 2) (6) . In all cases, the demethylated metabolites were found to be more active on cataplexy than were the parent compounds. We also found that the active dose of all anticataplectic compounds tested positively, correlated with the in vitro potency of each compound to the adrenergic transporter but not with that of the serotonergic transporter (6). In fact, the anticataplectic effects were negatively correlated with the in vitro potency for serotonergic reuptake inhibition, but this may be a bias since potent adrenergic reuptake inhibitors included in the study have a relatively low affinity to serotonergic reuptake sites. Although most of these results were obtained from inbred hypocretin receptor 2 (*hcrtr 2)*-mutated narcoleptic Dobermans, similar findings (the preferential involvement of adrenergic system) have been also obtained in more diverse cases of sporadic canine narcolepsy in various breeds donated to our colony (18).

The fact that serotonergic reuptake blockers, also known to have inhibitory effects on REM sleep, have less or no effect on cataplexy is surprising. Like adrenergic cells of the LC, serotonergic cells of the raphe nuclei dramatically decrease their activity during REM sleep (9). This discrepancy could be explained by a preferential effect of serotonergic projections on REM sleep features other than atonia, for example in the control of eye movements. In this model, adrenergic projections may be more important than serotonergic transmission in the regulation of REM sleep atonia and thus cataplexy (16). In favor of this hypothesis, a recent experiment has shown that serotonergic activity does not decrease during cataplexy in narcoleptic canines (19).

D. Neuroreceptor Subtypes Involved in the Control of Cataplexy

In order to dissect receptor subtypes that significantly modify cataplexy, more than 200 compounds with various pharmacological properties (cholinergic, adrenergic,

dopaminergic, serotonergic, prostaglandins, opioids, benzodiazepines, GABA-ergics and adenosinergics) have also been studied in the narcoleptic canine model (see Ref. 1 for details). Although many compounds (such as M2 antagonists, alpha-1 agonists, alpha-2 antagonists, dopaminergic D2/D3 antagonists, 5HT1a agonists, TRH analogs, prostaglandin E2, and L-type Ca^{2+} channel blockers) reduce cataplexy, very few compounds significantly aggravate cataplexy (cataplexy aggravating effects are assumed to be more specific, since cataplexy cane be non-specifically reduced by unpleasant drug side-effects) (1). Among the monoaminergic receptors, blockade of the postsynaptic adrenergic alpha-1b receptors (20) and stimulation of presynaptic alpha-2 autoreceptors (21) was also found to aggravate cataplexy, a result consistent with a primary adrenergic control of cataplexy. We also found that small doses of DA D2/D3 agonists significantly aggravated cataplexy and induced significant sleepiness in these animals (22,23). The cataplexy-inducing effects of D2/D3 agonists are, however, difficult to reconcile considering the fact that dopaminergic reuptake blockers (in contrast to adrenergic reuptake inhibitors) have absolutely no effect on cataplexy (16). We also found recently that sulpiride (a D2/D3 antagonist) significantly suppresses cataplexy in the canine model but has no effect on REM sleep (13). D2/3 agonists are clinically used for the treatment of human periodic leg movements during sleep (PLMS). Incidence of PLMS in high in human narcolepsy, and it also occurs in narcoleptic Doberman (24). The dopaminergic system (i.e., D2/D3 receptor mechanisms) may thus be specifically involved in sleep related motor control than REM sleep.

The sites of action of D2/D3 agonists were also investigated by local drug perfusion experiments, and a series of experiments identified acting sites for these compounds. These include dopaminergic nuclei or cell groups, such as the ventral tegmental area (23), substantia nigra (25) and A11 (26) (a diencephalic DA cell group that directly project to the spinal ventral horn), suggesting a direct involvement of DA cell groups and DA cell body autoreceptors for the regulation of cataplexy.

The mechanism for emotional triggering for cataplexy remains to be studied, but it is possible that multiple brain sites and multiple functional and anatomical systems are involved.

IV. Pharmacological Treatment of Cataplexy in Humans

A. Tricyclic Antidepressants

Since the 1960s, it has been known that imipramine is very effective for reducing cataplexy (Fig. 2) (27). Together with protriptyline and clomipramine, these tricyclic antidepressants are now the most commonly used anticataplectic agents (Table 3). Other antidepressant compounds of the tricyclic family have also been used with some success (Table 3).

Similar to the situation seen in patients with depression, the use of tricyclic antidepressants in the treatment of cataplexy is also hampered by a number of problems. The first is the relatively poor side-effect profile of most tricyclic compounds. These are mostly due to their anticholinergic properties, thus leading to dry mouth (and associated dental problems), tachycardia, urinary retention, constipation, and blurred vision (see Table 3). This profile was initially thought to be involved in anticataplectic

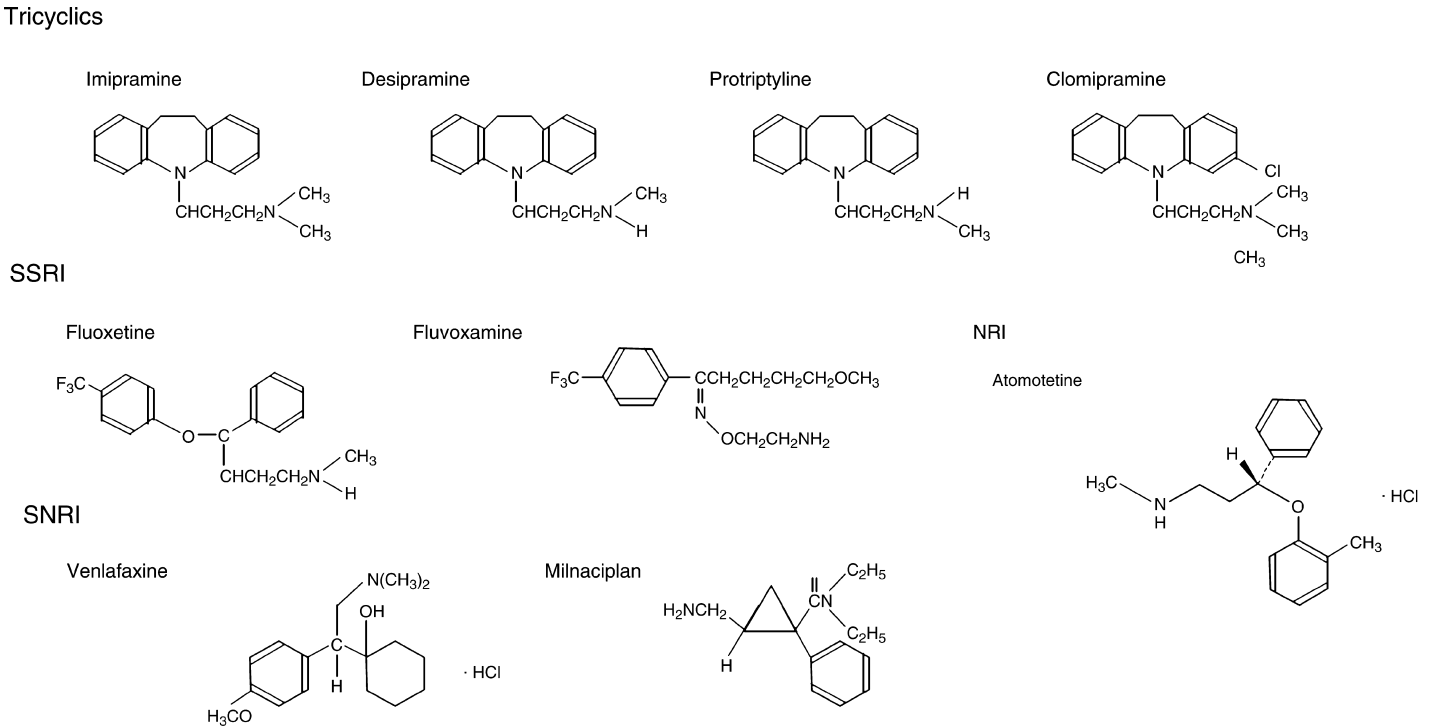


Figure 2 Chemical structures for tricyclics, selective sertonin (SSRI), serotonin and norepinephrine (SNRI), and norepinephrine (NRI) antidepressants.

Table 3 Antidepressants Currently Used as Anticataplectic Agents

Antidepressant compound	Usual daily doses	Half-life (hours)	Notes/Side-Effects
Tricyclics:			
Imipramine	10–100 mg	5–30	Dry mouth, anorexia, sweating, constipation, drowsiness
Desipramine	25–200 mg	10–30	(NE ≫ 5-HT > DA) a desmethyl metabolite of imipramine, effects and side effects similar to those of imipramine
Protryptiline	5–60 mg	55–200	(NE > 5-HT > DA) reported to improve vigilance measures anticholinergic effects
Clomipramine	10–150 mg	15–60	(5-HT > NE ≫ DA) Digestive problem, dry mouth, sweating, tiredness, impotence. Anticholinergic effects. Desmethyl-chlomipramine (NE ≫ 5-HT > DA) is an active metabolite
SSRIs:			
Fluoxetine	20–60 mg	24–72	No anticholinergic or antihistaminergic effects good anticataplectic effect but less potent than clomipramine. Active metabolite norfluoxetine has more adrenergic effects
Fluvoxamine	50–300 mg	15	No active metabolite, pharmacological profile similar to fluoxetine less active than clomipramine, gastrointestinal side effects
SNRIs:			
Venlafaxine	150–375 mg	4	New serotonergic and adrenergic uptake blocker; no anticholinergic effects, effective on cataplexy and sleepiness, nausea
Milnaciplan	30–50 mg	8	New serotonergic and adrenergic uptake blocker; no anticholinergic or antihistaminergic effects, effective on cataplexy
NRI:			
Atomotetine	40–60 mg[a]	5.2	Normally indicated for attention deficit hyperactivity disorder (ADHD)

SSRI: selective serotonin reuptake blocker; NSRI: norepinephrine/serotonin reuptake inhibitor; NRI: norepinephrine reuptake inhibitor. Reboxetine is another NRI, but is not available in the USA.

[a]Doses for treatments for ADHD; it is suggested to start with smaller doses for anticataplectic treatment. Gamma hydroxybutyric acid (GHB, sodium oxybate) is also reported to be effective on cataplexy and may act via GABA-B or via specific GHB receptors. Reduces dopamine release.

effects of these tricyclics. However, animal experiments as well as findings with newer antidepressants in human narcolepsy suggest that this property is not required for the anticataplectic property of antidepressants. Additional side-effects are weight gain, sexual dysfunction (impotence and/or delayed orgasm), tremors, antihistamine effects leading to sedation, and occasionally orthostatic hypotension due to the alpha-1 adrenergic blockade of some compounds. Nighttime sleep might also become more disturbed due to increased muscle tone and leg movements (28).

As described earlier, the cardinal pharmacological property of tricyclic antidepressants is their ability to inhibit the reuptake of NE (and epinephrine) and 5-HT (4). The degree of reuptake inhibition of NE and 5-HT is quite variable depending on the compound and on the existence of active metabolites (mostly active on adrenergic reuptake) (see Table 2). Active metabolites often have longer half-lives and tend to accumulate during chronic drug administration. The metabolic half-lives of these compounds also vary among individuals. Additionally, some tricyclic compounds (such as protriptyline), are also weak dopamine reuptake inhibitors (4). Thus, it is not simple to conclude which pharmacological property is important for the anticataplectic effects in humans.

B. Second Generation Antidepressants

The introduction of newer antidepressants with selective serotonergic reuptake inhibition properties and no anticholinergic effects (i.e., SSRI), (such as fluoxetine, fluvoxamine, paroxetine, sertraline, femoxamine, zimelidine and citalopram), has raised hope that the control of cataplexy can be achieved with fewer side-effects but, in general, clinicians have been less impressed with the anticataplectic effects of the SSRI than classical tricyclics (29,30). Among these compounds, fluoxetine at the 20 to 60 mg dose is a viable alternative to tricyclic compounds (29). Fluoxetine has a good side effect profile and induces anorexia rather than weight gain, a significant advantage for some patients.

Another, venlafaxine, is marketed as a 5-HT and NE reuptake inhibitor (SNRI) antidepressant, and it reduced cataplexy and sleepiness in a small pilot study. Another SNRI milnacipran is available in some European countries and Japan, and this compound reduces cataplexy in human and canines (experiments in progress). It is therefore interesting to evaluate the anticataplectic effects of selective NE blockers, such as atomoxetin and roboxetine in humans (no published data are yet available).

Because the newer second generation drugs usually have lower affinities for above-mentioned receptors, they are less likely to cause the adverse effects commonly seen with the older antidepressants (7).

C. MAOIs

MAOIs are known to potently reduce REM sleep, and are therefore candidate anticataplectic agents. This has led several investigators to use MAOIs for the treatment of narcolepsy (31,32). The extracellular effect of naturally released catecholamine is terminated by either reuptake or enzymatic degradation either by MAO and/or catechol-*O*-methyl transferase. MAO is a flavin-containing dominating enzyme located in the outer membranes of neural and glial mitochondria (5). It exists as two forms: MAO-A, blocked by clorgyline and with high affinity for noradrenaline and serotonin, and MAO-B, insensitive to clorgyline and with high affinity for phenylethylamine and dopamine (5).

Even if these compounds are clearly active on narcolepsy (31,32) and may be useful in cases refractory to more conventional treatment, the first generation of MAOIs are rarely used in clinical practice to date due to their poor safety profile (e.g., the "cheese effect") (see Ranga and Krishnan, 1994). It is also dangerous to use other drugs with sympathomimetic effects (tricyclic antidepressants, amphetamine-like compounds or simply catecholaminergic cardiac stimulants) in patients treated with MAOIs due to the existence of sometimes fatal interactions impossible to predict (5). Other side-effects include edema, impotence, weight gain, insomnia, long-term hypertension, and psychological disturbances (5). Drug withdrawal may lead to REM sleep rebound with exacerbation of the narcolepsy and the development of vivid nightmares (31). In addition, the first generation of MAOI (irreversible MAOIs) had the unique property of binding covalently to the active site of the enzyme ("suicide substrate"), thus leading to long-term (up to several weeks) enzymatic inhibition even after a single dose (5).

A safer generation of MAOIs is now becoming available. These include compounds with selective MAO-A or MAO-B inhibition and/or a reversible enzymatic inhibition profile. In contrast to irreversible MAOIs, reversible MAOIs are substrates for the MAOs and compete with the endogenous monoamines (5). Some of these new reversible MAOIs (brofaromine, moclobemide) are now being used in clinical trials in Europe and seem to be effective and safe for the treatment of human narcolepsy without noticeable side effects (33). These compounds can be used with minimal dietary precautions.

Selegiline is a potent irreversible MAO-B selective inhibitor used in the treatment of Parkinson's disease. This compound is essentially a methamphetamine derivative and is metabolized very significantly into amphetamine and methamphetamine (9–30% and 20–60% are found in urine respectively). The use of low doses of selegiline does not require dietary restriction. Ten milligrams of selegiline daily has no effect on the symptoms of narcolepsy, but 20 to 30 mg improves alertness and mood, and reduces cataplexy somewhat, an effect comparable to amphetamine at the same dose (34). Indeed, an experiment in canines also suggests that the anticataplectic effects of selegiline are likely to be explained by its active metabolites, L-amphetamine and L-methylamphetamine (35).

D. GHB

Gamma-hydroxybutyrate (GHB) or sodium oxybate, taken in the evening and once again during the night, reduces cataplectic attacks and other manifestations of REM sleep (36,37). Its elimination half-life is one to two hours. GHB increases NREM sleep stages 3 and 4, decreases nighttime awakenings, and consolidates fragmented REM sleep (37,38). Recent studies have demonstrated a measurable improvement in patients' reported daytime sleepiness and a moderate reduction in cataplexy. Although improvement in sleepiness occurs relatively quickly, anticataplectic effects appeared one to two weeks after the initiation of the treatment. GHB is reported to possess inhibitory effects through GABAB receptors, but the precise mechanisms of action of GHB on sleepiness and cataplexy are unknown. GHB given at bedtime and with a second dosage upon awakening during the night (at least 3 hours before rising time) may also help consolidate nocturnal sleep. If the patient wakes up during the night, he

may experience dizziness and confusional states. One problem with GHB is the non-medical use of this compound to elicit an altered state of consciousness with important social and legal implications (It had been classified as a Schedule I controlled substance in the USA). Its use for treating narcolepsy has been recently approved by the FDA (reclassified as a Schedule III controlled substance).

V. Treatment of Sleep Paralysis and Hypnagogic Hallucinations

The treatment of these two symptoms is much less well codified. Hypnagogic hallucinations can be quite bothersome, and often occur in patients who also suffer from frequent nightmares. As they are a manifestation of sleep onset REM sleep, the compounds that suppress REM sleep are usually helpful in alleviating this symptom, and tricyclic antidepressant treatment has been reported to have some beneficial effects (39). Sleep paralysis only rarely requires treatment, but tricyclic antidepressants are also very effective for preventing this symptom. Recently, high doses (60 mg qd) of fluoxetine have been advocated as a very active treatment for isolated sleep paralysis (40). GHB is also effective in suppressing both hypnagogic hallucinations and sleep paralysis (37).

VI. Hypocretin Agonists as Potential Therapeutic Agents

Since the occurrence of cataplexy in humans is tightly associated with hypocretin ligand deficiency, hypocretin replacement may be a new attractive treatment choice in human narcolepsy.

The effects of hypocretin-1 (hypocretin-2 has much lower in vivo biological activity, possible due its biological instability) administration on sleep and narcolepsy symptoms have been evaluated (41,42). Central administration of hypocretin-1, for example in the ventricle of wild type rodents or normal canines, is strongly wake-promoting (42). The effect is likely to be at least in part mediated by the hcrtr 2 as intracerebroventricular hypocretin-1 at the same dose (10–30 nmoles) did not promote wakefulness in *hcrtr 2 mutated* narcoleptic canines (42). A lack of wake-promoting effects of hypocretin-1 was also observed in *hcrtr 2 KO* mice (Mieda et al., personal communication).

Experiments conducted after intravenous administration of hypoocretin-1 have been performed in *hcrtr 2 mutated* canines and in two hypocretin ligand deficient narcoleptic dogs. In spite of a previous report (41), we were unable to detect any significant effect even at very high doses of hypocretin-1 in *hcrtr-2 mutated* animals (42). This result was not surprising considering the lack of effects after central administration of the same dose in these animals lacking hcrtr 2 (see above). More interestingly, a possible very slight and short lasting suppression of cataplexy was observed in a single hypocretin deficient narcoleptic animal at extremely high intravenous doses (42,43). Intrathecal administration of hypocretin-1 by implanting a Medtronic pump with catheterization of the cisterna magna was also carried out in a single hypocretin deficient narcoleptic canine (43). However, no significant effect on cataplexy was

observed (43), probably because the hypocretin-1 did not diffuse into upper ventricular compartments. Additional studies using intraventricular rather than intracisternal injections will be needed to verify that hypocretin deficient narcoleptic canines are responsive to supplementation.

Using hypocretin neuron-ablated narcoleptic mice (*orexin/ataxin 3* transgenic mice), Mieda et al. recently demonstrated that replacement of central hypocretin by either pharmacological (intracerebroventricular injection of hypocretin-1) or genetic (ectopic expression of hypocretin in the brain) manipulations allowed for the rescue of the narcolepsy–cataplexy phenotype (44). These results indicate that hypocretin neuron–ablated mice retain the ability to respond to hypocretin neuropeptides and that hypocretin receptors, intracellular signaling, postsynaptic neural networks, and other downstream neurotransmitter pathways remain anatomically and functionally intact. Most importantly, a temporally regulated and spatially targeted secretion of hypocretin is not likely to be necessary to prevent narcoleptic symptoms (44). The potential treatment of narcolepsy–cataplexy symptoms in humans by using agonists for hypocretin receptors is a promising new field. Developments of small molecular weight, nonpeptide agonists will be essential for this purpose.

VII. Conclusion

Cataplexy is currently treated with antidepressants, a class of compounds that enhance monoaminergic neurotransmission by inhibition of monoamine reuptake (NE, 5HT, DA). Most narcolepsy-cataplexy patients also take wake-promoting compounds, but these have little effect on cataplexy. Experiments in canine narcolepsy suggest a preferential involvement of NE than 5HT reuptake inhibition in anticataplectic properties of drugs. DA reuptake inhibition does not reduce cataplexy, but does significantly enhance wakefulness. In humans, compounds with NE reuptake inhibition also reduce cataplexy. SSRIs are also very commonly used as anticataplectics in human. This is mostly due to their better side-effect profiles, but the anticataplectic effects of these compounds are rather modest. Recently selective NE and NE/5-HT reuptake inhibitors were introduced, and evaluations of these are in progress and may bring profound beneficial effects.

MAOIs are another choice of anticataplectic medication, but using classical, nonselective, irreversible MAOIs are hampered by various side effects, including hypertensive crisis. Reversible and selective MAOIs are available is some countries, and this may be useful for some severe or refractory cataplexy cases.

Finally, hypocretin replacement therapy may be more straightforward and efficient for the treatment of both cataplexy and sleepiness, but the development of small molecular weight peptide agonists is essential. If these are effective in humans, cell transplantation and/or gene therapy may also be developed in near future.

Acknowledgments

The author acknowledges Dr. Nobuhiro Fujiki for the artwork and Ms. Mali Einen for editing the manuscript.

References

1. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol 1997; 52:27–78.
2. Nishino S. The hypocretin/orexin system in health and disease. Biol Psychiatry 2003; 54:87–95.
3. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002; 59:1553–1562.
4. Nelson JC. Trycyclic and tetoracyclic drugs. In: Schatzberg AF, Nemeroff CB, eds. Textbook of Psychopharmacology. Washington, DC: American Psychiatric Press, 2004:207–246.
5. Krishnan KRR. Monoamine Oxidase inhibitors. In: Schatzberg AF, Nemeroff CB, eds. Textbook of Psychopharmacology. Washington, DC: American Psychiatric Press, 2004:303–314.
6. Nishino S, Arrigoni J, Shelton J, Dement WC, Mignot E. Desmethyl metabolites of serotonergic uptake inhibitors are more potent for suppressing canine cataplexy than their parent compounds. Sleep 1993; 16:706–712.
7. Rosenbaum JF, Tollfson AD. Fluoxetine. In: Schatzberg AF, Nemeroff CB, eds. Textbook of Psychopharmacology. Washington, DC: American Psychiatric Press, 2004:231–246.
8. Nishino S, Riehl J, Hong J, Kwan M, Reid M, Mignot E. Is narcolepsy REM sleep disorder? Analysis of sleep abnormalities in narcoleptic Dobermans. Neuroscience Research 2000; 38:437–446.
9. Siegel JM. Brainstem mechanisms generating REM sleep. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. Philadelphia: W. B. Saunders Company, 2000:112–133.
10. Nishino S, Tafti M, Reid MS, et al. Muscle atonia is triggered by cholinergic stimulation of the basal forebrain: implication for the pathophysiology of canine narcolepsy. J Neurosci 1995; 15:4806–4814.
11. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 1997; 20:620–629.
12. Guilleminault C. Cataplexy. Narcolepsy (Advances in Sleep Research Vol. 3) 1976:125–143.
13. Okura M, Riehl J, Mignot E, Nishino S. Sulpiride, a D2/D3 blocker, reduces cataplexy but not REM sleep in canine narcolepsy. Neuropsychopharmacology 2000; 23:528–538.
14. Nishino S, Taheri S, Black J, Nofzinger E, Mignot E. The neurobiology of sleep in relation to mental illness. In: Charney DS NE, ed. Neurobiology of Mental Illness. New York: Oxford University Press, 2004:1160–1179.
15. Wu MF, Gulyani SA, Yau E, Mignot E, Phan B, Siegel JM. Locus coeruleus neurons: cessation of activity during cataplexy. Neuroscience 1999; 91:1389–1399.
16. Mignot E, Renaud A, Nishino S, Arrigoni J, Guilleminault C, Dement WC. Canine cataplexy is preferentially controlled by adrenergic mechanisms: evidence using monoamine selective uptake inhibitors and release enhancers. Psychopharmacology 1993; 113:76–82.
17. Peet M, Coppen A. The pharmacokinetics of antidepressant drugs: relevance to their therapeutic effect. In: Paykel ES, Coppen A, eds. Psychopharmacology of Affective Disorders. Oxford: Oxford University Press, 1979:91–107.
18. Baker TL, Dement WC. Canine narcolepsy-cataplexy syndrome: evidence for an inherited monoaminergic-cholinergic imbalance. In: McGinty DJ, Drucker-Colin R, Morrison A, Parmeggiani PL, eds. Brain Mechanisms of Sleep. New York: Raven Press, 1985:199–233.
19. Wu MF, John J, Boehmer LN, Yau D, Nguyen GB, Siegel JM. Activity of dorsal raphe cells across the sleep-waking cycle and during cataplexy in narcoleptic dogs. J Physiol 2004; 554:202–215.
20. Nishino S, Fruhstorfer B, Arrigoni J, Guilleminault C, Dement WC, Mignot E. Further characterization of the alpha-1 receptor subtype involved in the control of cataplexy in canine narcolepsy. J Pharmacol Exp Ther 1993; 264:1079–1084.
21. Nishino S, Haak L, Shepherd H, et al. Effects of central alpha-2 adrenergic compounds on canine narcolepsy, a disorder of rapid eye movement sleep. J Pharmacol Exp Ther 1990; 253:1145–1152.
22. Nishino S, Arrigoni J, Valtier D, et al. Dopamine D2 mechanisms in canine narcolepsy. J Neurosci 1991; 11:2666–2671.
23. Reid MS, Tafti M, Nishino S, et al. Local administration of dopaminergic drugs into the ventral tegmental area modulate cataplexy in the narcoleptic canine. Brain Res 1996; 733:83–100.
24. Okura M, Fujiki N, Ripley B, et al. Narcoleptic canines display periodic leg movements during sleep. Psychiatry Clin Neurosci 2001; 55:243–244.
25. Honda K, Riehl J, Mignot E, Nishino S. Dopamine D3 agonists into the substantia nigra aggravate cataplexy but do not modify sleep. NeuroReport 1999; 10:3717–3724.

26. Okura M, Fujiki N, Kita I, et al. The roles of midbrain and diencephalic dopamine cell groups in the regulation of cataplexy in narcoleptic Dobermans. Neurobiol Dis 2004; 16:274–282.
27. Akimoto H, Honda Y, Takahashi Y. Pharmacotherapy in narcolepsy. Dis Nerv Sys 1960; 21:704–706.
28. Thorpy MJ, Goswami M. Treatment of narcolepsy. In: Thorpy MJ, ed. Handbook of Sleep Disorders. New York: Marcel Dekker, Inc., 1990:235–258.
29. Langdon N, Shindler J, Parkes JD, Bandak S. Fluoxetine in the treatment of cataplexy. Sleep 1986; 9:371–373.
30. Montplaisir J, Godbout R. Serotonergic reuptake mechanisms in the control of cataplexy. Sleep 1986; 9:280–284.
31. Wyatt RJ, Fram DH, Buchbinder R, Snyder F. Treatment of intractable narcolepsy with a monoamine oxidase inhibitor. N Engl J Med 1971; 285:987–991.
32. Gillin JC, Horwitz D, Wyatt RJ. Pharmacologic studies of narcolepsy involving serotonin, acetylcholine, and monoamine oxidase. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy (Advances in Sleep Research Vol. 3). New York: Spectrum Publications, 1976:585–603.
33. Hohagen F, Mayer G, Memche A, et al. Treatment of narcolepsy-cataplexy syndrome with the new selective and reversible mao-a inhibitor brofaromine–a pilot study. J Sleep Res 1993; 2:250–256.
34. Hublin C, Partinen M, Heinonen EH, Puukka P, Salmi T. Selegiline in the treatment of narcolepsy. Neurology 1994; 44:2095–2101.
35. Nishino S, Arrigoni J, Kanbayashi T, Dement WC, Mignot E. Comparative effects of MAO-A and MAO-B selective inhibitors on canine cataplexy. Sleep Res 1996; 25:315.
36. Broughton R, Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. Can J Neurol Sci 1979; 6:1.
37. Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with g-hydroxybutyrate. A review of clinical and sleep laboratory findings. Sleep 1986; 9:285–289.
38. Broughton R, Mamelak M. Effects of nocturnal gamma-hydroxybutyrate on sleep/waking patterns in narcolepsy-cataplexy. Le J Can Sci Neurol 1980; 7:23.
39. Takahashi S. The action of tricyclics (alone or in combination with methylphenidate) upon several symptoms of narcolepsy. In: Guilleminault C, Dement WC, Passouant P, eds. Narcolepsy. New York: Spectrum Publication, 1976:625–638.
40. Koran L, Raghavan S. Fluoxetine for isolated sleep paralysis. Psychomatics 1993; 34:184–187.
41. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Research Online 2000; 3:23–28.
42. Fujiki N, Ripley B, Yoshida Y, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin ligand deficient narcoleptic dog. Sleep 2003; 6:953–959.
43. Schatzberg SJ, Barrett J, Cutter Kl, Ling L, Mignot E. Case Study: Effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. J Vet Internal Med 2004; 18:586–588.
44. Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci USA 2004.

50
Modafinil: Mechanisms of Action

THOMAS E. SCAMMELL
Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, U.S.A.

I. Introduction

Over just a few years, modafinil has become the most popular medication for treating the daytime sleepiness of narcolepsy. Its' safety and efficacy are well established, but its' mechanism of action remains poorly understood. This chapter summarizes the neuropharmacology of modafinil, and outlines how research on this clinically important drug has shed light on the basic neurobiology of sleep and wakefulness.

Modafinil is 2-[(diphenylmethyl)-sulfinyl]acetamide (Figure 1). This drug has no chemical similarity to stimulants such as cocaine or pemoline, and has only slight similarity to amphetamine (AMPH). Compared to AMPH, modafinil has an additional phenyl group, a sufinyl group, and an amide instead of an amine group. Though it is difficult to predict function from structure, these differences suggest that modafinil may have a distinct mechanism of action.

II. Neurobiology of Wakefulness

To understand how modafinil promotes wakefulness, it is helpful first to review some of the basic pathways that promote arousal [for review, see (1)]. Neurons of the laterodorsal and pedunculopontine tegmental nuclei use acetylcholine to activate thalamocortical signaling, and this activation of the cortex is further reinforced by direct projections from the basal forebrain. Amines such as norepinephrine (NE), serotonin (5-HT), and histamine act directly on cortical and subcortical regions to promote wakefulness. The locus coeruleus is the major source of forebrain NE, and may particularly increase cortical activation during times of stress or vigilance (2,3). The tuberomammillary nucleus is the sole source of histamine within the CNS. Mice lacking histamine are slower to wake at the beginning of their active period (4), and the tuberomammillary neurons remain active during cataplexy (5), suggesting that histamine may help initiate or maintain consciousness. Serotonin is released by neurons of the raphe nuclei and has numerous effects on mood and alertness.

In contrast to these other aminergic systems, researchers have often underestimated the importance of dopamine (DA) in promoting wakefulness. Most likely, this is because the firing rates of many DA-producing neurons do not obviously vary with behavioral

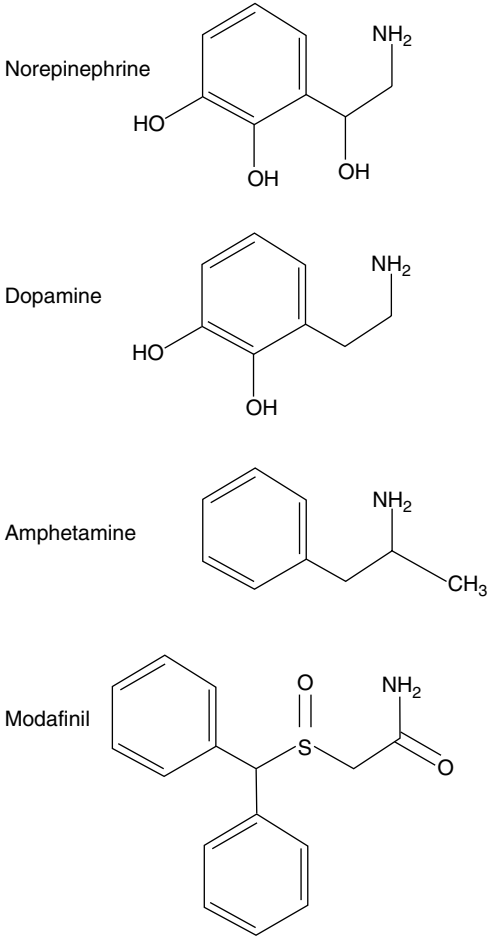


Figure 1 Chemical structures of norepinephrine, dopamine, amphetamine, and modafinil. Like norepinephrine and dopamine, amphetamine consists of a short carbon chain with a phenyl group on one end and an amine group on the other. Modafinil has some similarities but has an additional phenyl group, a sulfonyl group, and an amide group.

state whereas other aminergic regions are clearly more active during wakefulness. In addition, DA is produced by many different cell groups, and it remains to be determined just which of these promote wakefulness. Nevertheless, DA release is greatest during wakefulness (6), and lesions that include the dopaminergic neurons of the ventral tegmental area reduce behavioral arousal (7). Recent work has also identified a wake-active population of dopamine-producing neurons in the ventral periaqueductal grey that project to other arousal regions and appear to be necessary for normal wakefulness (8). People with DA deficiency from Parkinson's disease are often sleepy, and dopamine antagonists are frequently sedating. This physiologic and clinical evidence clearly demonstrates the importance of DA in generating wakefulness.

Wakefulness is a complex state that is always changing in response to variations in the environment and internal milieu. Most likely, each of these arousal systems is active during specific times and promotes particular aspects of wakefulness. Full wakefulness requires coordinated activity in all these arousal systems.

III. Amphetamines

Amphetamines are the prototypical wake-promoting drugs, and understanding their neurobiology provides a useful foundation for understanding modafinil.

AMPH and related drugs increase signaling by dopamine, norepinephrine, and to a lesser extent, serotonin. These transmitters are normally recycled back into nerve terminals by selective transporters for DA, NE, and 5-HT (the DAT, NET, and SERT, respectively) (9). At low doses, AMPH competes with DA and NE at the DAT and NET, profoundly inhibiting their reuptake (10). Once AMPH binds to the DAT and NET, it is transported into the nerve terminal (11), and when the transporters then return to their outward orientation, DA and NE can move out through the transporters in a process of exchange diffusion (12). In addition, AMPH causes internalization of the DAT, further hindering the uptake of DA (13). This inhibition of reuptake and reverse transport of DA are the main effects of AMPH at typical clinical doses (14).

Moderate doses of AMPH also increase aminergic signaling through an additional mechanism. Monoamines are pumped into synaptic vesicles by the vesicular monoamine transporter (VMAT2). AMPH hinders the packaging of amines by reducing the level of VMAT2 (15) and by disrupting the concentration of monoamines in vesicles (16, 17). Specifically, AMPH enters vesicles through VMAT2 (18) or diffusion, and because the interior of synaptic vesicles is acidic, the amine group on AMPH becomes protonated to form an ammonium ion. These charged molecules accumulate inside the vesicles, eventually disrupting the proton gradient that is necessary for the sequestration of monoamines (19). The resulting high cytoplasmic concentrations of DA and NE produce an even greater efflux of these neurotransmitters out through the membrane transporters.

Lastly, high doses of AMPH inhibit monoamine oxidase A, thus slowing the degradation of monoamines and further increasing their synaptic concentrations (20). Through these synergistic effects, AMPH produces persistently high synaptic concentrations of DA and NE, even when DA- and NE-producing neurons are physiologically inactive.

Nishino, Mignot, and colleagues proposed that the wake-promoting efficacy of AMPH and related drugs is mainly due to an increase in DA signaling (21). The potency with which these drugs increase wakefulness is proportionate to their affinity for the DAT (21). In addition, DAT knockout mice have no increase in wakefulness with methamphetamine (22). While enhanced NE signaling may play some role, drugs that selectively inhibit the NET are not very effective at promoting wakefulness, and lesions of the NE-producing neurons of the LC do not alter the response to AMPH (23). In addition, AMPH still increases locomotor activity (and probably wakefulness) in NET knockout mice (24). Thus, although AMPH can alter NE and 5-HT signaling, increased DA signaling probably plays the greatest role in the wake-promoting effects of these drugs.

IV. Modafinil's Mechanisms of Action

A. Mechanisms that Now Appear Improbable

Since its discovery over 20 years ago, many mechanisms have been proposed to account for modafinil's effects. Several of these theories have been undermined by recent evidence, but they still provide useful perspectives.

1) Is modafinil an α agonist? Early research suggested that modafinil might stimulate α adrenergic receptors (25). In cats, modafinil-induced wake was attenuated by the α1 antagonist prazosin (26). However, modafinil does not bind to α1 receptors (27) nor does it produce smooth muscle contraction in a vas deferens preparation as seen with other direct sympathomimetics. In fact, modafinil does not bind to receptors for DA, 5-HT, or acetylcholine (27). The hyperlocomotion produced by AMPH largely depends on the α1b receptor because mice lacking this receptor have much less hyperactivity with AMPH (28). Increased signaling through this receptor with modafinil appears unlikely because modafinil does not increase locomotor activity beyond that seen with normal wakefulness (29,30). These preclinical observations generally argue against a direct effect of modafinil on a receptors.

Clinical observations provide the strongest evidence that modafinil is not a direct or indirect sympathomimetic. By increasing NE signaling, AMPH causes dilation of the pupils, but modafinil has no effect on the pupils (31). Some studies have noted slight increases in heart rate or blood pressure with high doses of modafinil (32–38). However, these changes were small and many other clinical studies of modafinil have found no changes in heart rate or blood pressure (31,39–43). In the most comprehensive perspective on this issue, no changes in heart rate or blood pressure were seen in a meta-analysis of seven large clinical trials of modafinil (1,501 patients in total) (44). These clinical observations suggest that at the usual clinical doses, modafinil does not simply increase adrenergic signaling.

2) Does modafinil directly alter glutamate or GABA signaling? In a series of studies, Fuxe and colleagues investigated whether modafinil altered glutamate and GABA signaling. In unanesthetized animals, they showed that modafinil increased the extracellular concentration of glutamate and decreased that of GABA in cortex, striatum, medial preoptic area, and posterior hypothalamus (45–48). However, subsequent in vitro studies by the same group found that modafinil had no direct effect on the reuptake of glutamate or the synthesis of GABA or glutamate (48,49). Therefore, they suggested that the changes in GABA and glutamate signaling are probably a consequence of altered activity in aminergic systems (50,51). In fact, the observed changes are similar to those seen during spontaneous wakefulness and may simply reflect the increase in wakefulness produced by modafinil.

3) Does modafinil require orexin/hypocretin? In mice and rats treated with modafinil, most orexin-producing neurons express Fos, an indicator of neuronal activity (52,53). Because the orexin neurons play such an essential role in the regulation of normal wakefulness, we hypothesized that modafinil might promote wake by increasing orexin signaling (52). However, modafinil promotes wakefulness very effectively in many people with narcolepsy caused by a loss of the orexin neurons (54). In addition, modafinil robustly increases wakefulness in orexin knockout mice and in dogs lacking ox2 receptors (29,55). Though enhanced orexin signaling may be one of many wake-promoting mechanisms engaged by modafinil, these observations demonstrate that orexin is not necessary.

B. Mechanisms that Appear Likely

Research on modafinil has indicated that it may act through many systems to promote wakefulness, and several studies have used Fos immunostaining to examine the broad patterns of neuronal activation induced by modafinil. Lin and colleagues treated cats with modafinil and found that it activated neurons in the anterior hypothalamic area, a region adjacent to the suprachiasmatic nucleus (56). A similar pattern was later reported in modafinil-treated rats (57).

These initial studies used small numbers of animals and did not fully quantify the changes in Fos expression, so we treated rats with various doses of modafinil at different times of day (53). Modafinil-induced wakefulness was consistently associated with increased expression of Fos in wake-promoting hypothalamic brain regions including the orexin neurons and tuberomammillary nucleus. More cortical neurons contained Fos than seen in spontaneously awake rats. The striatum contained only rare Fos immunoreactive neurons in vehicle-treated rats, but modafinil dose-dependently increased Fos in this region as well as in part of the bed nucleus of the stria terminalis and the central nucleus of the amygdala (Figure 2). In our experiments, modafinil did not induce Fos in the anterior hypothalamic area or suprachiasmatic nucleus, though these areas were implicated in prior studies (56,57).

Activation of the tuberomammillary nucleus and orexin neurons in these experiments might reflect a direct effect of modafinil on these neurons, but it could easily be a consequence of wakefulness as these cells express Fos even during spontaneous

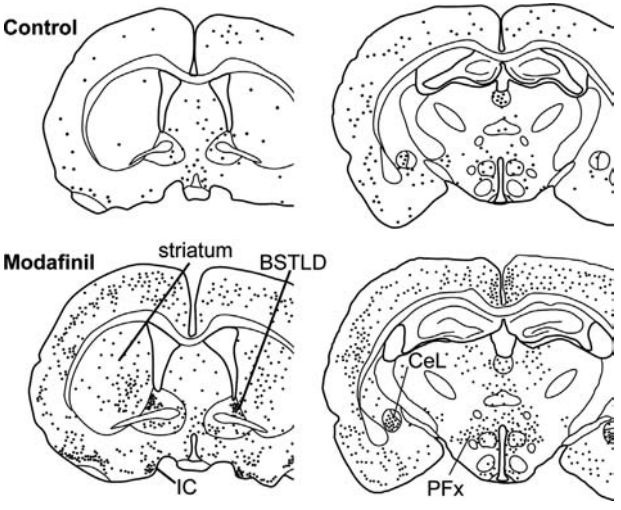


Figure 2 Modafinil induces Fos in many dopamine-receptive brain regions. Rats were treated with control vehicle or a low dose of modafinil (75 mg/kg) at midnight. A spontaneously awake, vehicle-treated rat has scattered Fos-immunoreactive neurons in the cortex, bed nucleus of the stria terminalis, and hypothalamus. A rat treated with modafinil has abundant Fos expression in dopamine-receptive areas such as the cortex, striatum, laterodorsal subnucleus of the stria terminalis (BSTLD), islands of Calleja (IC), lateral subnucleus of the central nucleus of the amygdala (CeL), and perifornical region (PFx). *Source*: Adapted from Ref. 53.

wakefulness (58,59). However, normal wakefulness is not associated with much Fos expression in the striatum, bed nucleus, or amygdala. All these areas receive moderate to substantial dopaminergic inputs, and Fos is induced in many of these regions by dopamine agonists or AMPH (56,60,61). Although the clinical side effects of modafinil differ substantially from those of AMPH, this pattern of Fos expression suggests that modafinil may enhance dopaminergic signaling.

1) *Does modafinil enhance DA signaling*? Several studies using microdialysis have shown that modafinil increases the extracellular concentration of DA. In rats, DA concentrations in the nucleus accumbens increase for about 2 hours after a moderate wake-promoting dose of modafinil (47) and a similar rise occurs in the cortex (62). Modafinil also briefly increases the concentration of DA in the striatum of narcoleptic dogs (22). One early study failed to detect any rise in DA or its metabolites (DOPAC and HVA) in mice, but this was probably because the dose of modafinil was too low (63).

This rise in extracellular DA may be caused by blockade of the DAT. Modafinil has nearly the same affinity for the DAT as dextroamphetamine in striatal tissue from dogs (3.8 μM) or guinea pigs (1.9 μM) (21, 27). Because wake-promoting concentrations of modafinil in brain are probably about 15 μM in humans and around 100 μM in rats (64) (and see Discussion in (65)), modafinil could easily bind to the DAT.

To test whether modafinil's wake-promoting effects depend on the DAT, Wisor and colleagues treated wild type and DAT knockout mice with modafinil partway through their normal sleep period (22). With a moderate dose of modafinil, wild type mice had a clear increase in wakefulness, but DAT knockout mice had no increase in wakefulness.

This important observation demonstrates that the DAT is necessary for modafinil's wake-promoting effects. Most likely, modafinil acts on the DAT to block the reuptake of DA, just as shown with AMPH (Figure 3). Alternatively, modafinil may enter nerve terminals through the DAT, and once inside, it may alter DA packaging or other processes. A third interpretation is that constitutive loss of DAT changes the neurobiology of these animals so severely that it is difficult to draw any conclusions: DAT KO mice have very low presynaptic concentrations of DA, very high extracellular concentrations of DA, down-regulation of pre- and post-synaptic DA receptors, and numerous behavioral abnormalities, including hyperactivity, more wakefulness, longer wake bouts, less NREM sleep, and hypersensitivity to caffeine (22,66). Nevertheless, the complete absence of a waking response to modafinil strongly suggests that the DAT plays an essential role.

Can inhibition of the DAT fully explain modafinil's effects? The selective DAT inhibitor GBR12909 produces only about half as much wakefulness as modafinil (22), and other DAT inhibitors such as mazindol and buproprion are only moderately effective at promoting wakefulness. Thus, modafinil may have additional effects that promote wakefulness.

2) *Does modafinil enhance NE signaling*? Most research on modafinil has focused on how it might increase the activity of arousal systems, but modafinil might promote wakefulness by reducing the activity of sleep-promoting neurons. We noticed that in modafinil-treated rats, Fos expression was reduced in the sleep-producing neurons of the ventrolateral preoptic area (VLPO) (53). VLPO neurons are inhibited by NE (67), and modafinil increases NE release in the medial hypothalamus (62).

Using preoptic brain slices, Gallopin and colleagues found that modafinil markedly decreased the firing rates of VLPO neurons (65). Modafinil did not directly inhibit

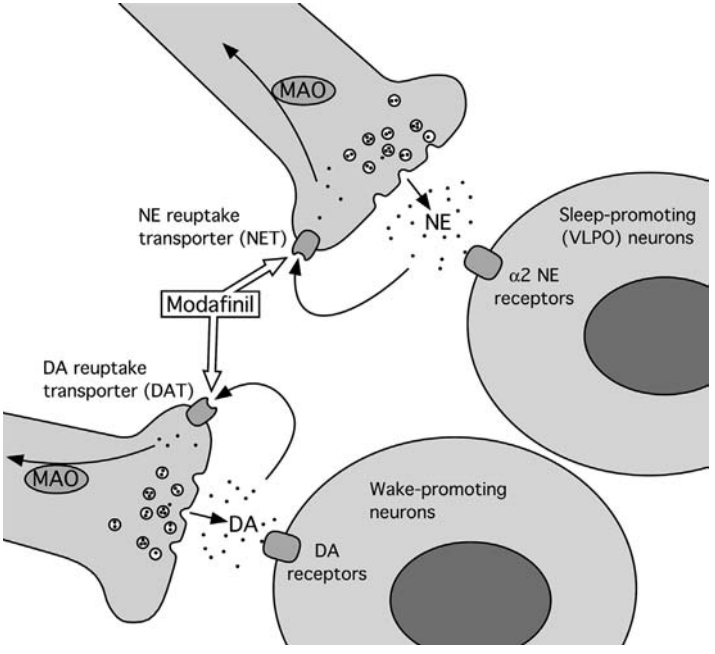


Figure 3 Modafinil may promote wakefulness by blocking the reuptake of dopamine (DA) and norepinephrine (NE). By blocking the DAT, modafinil may increase the activation of wake-promoting neurons by DA. In addition, modafinil may inhibit sleep by blocking the NET, thus increasing the inhibition of sleep-promoting ventrolateral preoptic (VLPO) neurons by NE. Note that in this model, modafinil does not alter the vesicular packaging of amines, and therefore, cytoplasmic concentrations of DA and NE are not elevated. Modafinil also does not inhibit monoamine oxidase (MAO).

these cells, but instead amplified and prolonged their inhibition by NE through $\alpha 2$ receptors. Modafinil does not bind to or alter signaling through $\alpha 2$ receptors (27, 65), but instead it appeared to inhibit the NET presynaptically much like nisoxetine. This observation was surprising because modafinil does not bind to the NET (27) or alter the firing of LC neurons (68). While it remains unclear how modafinil interacts with the NET, it may help stave off the transition into sleep by potentiating the inhibition of VLPO neurons by NE.

A straightforward interpretation of this observation is that modafinil promotes the release of NE in the VLPO region, and this high NE concentration inhibits sleep. However, the NET also is essential for the reuptake of DA, particularly in brain regions with low expression of DAT such as the preoptic area. Thus, by inhibiting the NET, modafinil could increase the extracellular concentrations of DA as well as NE.

Drugs that enhance NE signaling suppress cataplexy very effectively (69), but modafinil has no effect on cataplexy. Perhaps, the usual clinical doses of modafinil enhance NE signaling only to a small degree or only in specific brain regions.

While this important study demonstrates that modafinil can alter NE signaling, the functional importance of this observation has yet to be tested. Further testing

of modafinil in NET knockout mice or in mice with a selective depletion of NE in the preoptic area will be most helpful.

3) *Modafinil may act on additional aminergic systems*. Several studies have measured the release of other monoamines after treatment with modafinil. In unanesthetized rats, modafinil increased the extracellular concentration of 5-HT in frontal cortex, striatum, amygdala, and dorsal raphe (45,62,70,71). In anesthetized rats, modafinil (150 mg/kg) increased extracellular histamine concentrations by about 50%, but the rise occurred 3–4 hours after the injection (72)—much later than the usual onset of wakefulness by modafinil. Increased signaling by 5-HT and histamine certainly may contribute to modafinil's mechanism, but the importance of these neurotransmitters has yet to be determined.

V. Modafinil Differs from Amphetamine in Many Ways

This recent evidence suggests many similarities between the mechanisms of modafinil and AMPH, yet these drugs differ in many important ways (Table 1).

Modafinil lacks many of the behavioral features of AMPH. In rodents, even low doses of amphetamines can induce stereotyped behaviors such as compulsive licking, sniffing, grooming, or chewing, and these behaviors are typical of many drugs that

Table 1 Amphetamines and Modafinil Have Some Similarities and Many Differences[a]

	Amphetamines	Modafinil
Clinical observations		
Promotes wake	Yes	Yes
Increases sympathetic activity	Yes	Only at high dose
Nighttime insomnia	Common	Rare
Addictive potential	Yes	Minimal
Reduces cataplexy	Somewhat	No
Reduces food intake	Yes	Slightly
Basic observations		
Effect blocked by alpha-methylparatyrosine	Yes	No
Inhibits monoamine oxidase	Yes (at high dose)	No
Induces stereotypes	Yes	No
Rebound hypersomnia	Yes	No
Inhibits firing of locus coeruleus neurons	Yes	No
Binds to NE transporter	Yes	No
Binds to DA transporter	Yes	Yes
Increases extracellular DA	Yes	Yes
Waking effects blocked by haloperidol	Yes	No
Induces Fos in striatum	Yes	Yes
Induces Fos in accumbens nucleus	Yes	No
Promotes wake in DA transporter KO mice	No	No
Increases locomotor intensity	Yes	No
Increases body temperature	Yes	No

[a]See text for supporting references.

increase dopamine signaling. Though a very high dose of modafinil induced a slight increase in stereotypies in mice, normal wake-promoting doses produced none of these behaviors in rats or mice (25). In addition, methamphetamine-induced wakefulness is followed by several hours of intense rebound hypersomnia, but sleep is recovered more gradually with modafinil (30), and patients report less "crash" with modafinil than with AMPH.

Modafinil also differs from AMPH in its pharmacologic effects. Unlike amphetamines, modafinil does not inhibit monoamine oxidase so amines are metabolized at a normal rate (63). More importantly, modafinil probably has no effect on the packaging of amines in synaptic vesicles. Modafinil contains an amide group and probably does not disrupt vesicular packaging because it is unlikely to become protonated within synaptic vesicles like the amine group of AMPH. Reserpine produces bradykinesia and sedation by blocking the uptake of monoamines into synaptic vesicles, and this effect is reversed by AMPH but not by modafinil (73). Consequently, by increasing cytoplasmic concentrations of catecholamines, AMPH doubles the spontaneous release of DA. Because it probably does not disrupt monoamine packaging, modafinil is unlikely to substantially raise the cytoplasmic concentrations of these neurotransmitters.

In fact, this may be the pivotal difference between modafinil and AMPH. If modafinil just blocks the reuptake of amines but does not produce an efflux of amines, it may simply amplify physiologic amine signaling. In contrast, by triggering an efflux of amines that is independent of electrophysiologic activity, AMPH produces chronically high extracellular levels of amines.

This difference could explain several key observations. Lin and colleagues showed that AMPH does not increase wakefulness if the production of catecholamines is blocked by α-methylparatyrosine (α-MPT), but α-MPT has no effect on the response to modafinil (26). Possibly, this is because the synthesis of new catecholamines is substantially increased by AMPH but not modafinil. Dextroamphetamine inhibits the firing of neurons in the ventral tegmental area and locus coeruleus most likely through efflux of amines that stimulate autoinhibitory receptors (68). In contrast, a moderate dose of modafinil had no effect on the activity of these neurons. In addition, when taken in the evening, amphetamines hinder sleep, producing lighter, more fragmented sleep, probably because they induce the inappropriately timed release of catecholamines. However, even when taken near bedtime, modafinil at typical doses has very little effect on sleep (74–76) perhaps because dopamine signaling is minimal at this time, and blockade of the DAT would have little effect. Experiments to test the effects of modafinil on DA turnover, amine packaging, and cytoplasmic concentrations of amines should help define these key features of modafinil's mechanism.

VI. Conclusions

Though many details remain unclear, these experiments suggest a general mechanism through which modafinil may promote wakefulness (Figure 3). Modafinil may inhibit the reuptake of dopamine and norepinephrine, thus promoting wake at times when these neurotransmitters are normally released. During the sleep period, when amine release is minimal, modafinil has little effect, whereas by chronically releasing

catecholamines, amphetamines promote wake even at night. Quite possibly, modafinil may be effective because it enhances signaling by both DA and NE that can synergistically activate the forebrain while VLPO activity is suppressed by NE.

This model still requires some critical testing and many questions remain unanswered. Does modafinil simply inhibit the uptake of DA and NE or does it have effects within the cytoplasm after entering cells through these transporters? Does modafinil alter the vesicular packaging of monoamines? If modafinil enhances DA signaling, where are the dopaminergic neurons that promote wakefulness? Modafinil is an effective drug, but answering these questions should allow the design of even more potent wake-promoting agents.

Acknowledgments

Paul A. Rosenberg, Clifford B. Saper, and Arthur Seelig provided many thoughtful comments on this manuscript. This study was supported by NIH grants MH62589 and HL02013.

References

1. Espana RA, Scammell TE. Sleep neurobiology for the clinician. Sleep, 2004; 27(4):811–820.
2. Hunsley MS, Palmiter RD. Norepinephrine-deficient mice exhibit normal sleep-wake states but have shorter sleep latency after mild stress and low doses of amphetamine. Sleep, 2003; 26(5):521–526.
3. Aston-Jones G, Chen S, Zhu Y, Oshinsky ML. A neural circuit for circadian regulation of arousal. Nat Neurosci, 2001; 4(7):732–738.
4. Parmentier R., Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS JSAnatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. J Neurosci, 2002; 22(17):7695–7711.
5. John J, Wu MF, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron, 2004; 42(4):619–634.
6. Trulson ME. Simultaneous recording of substantia nigra neurons and voltammetric release of dopamine in the caudate of behaving cats. Brain Res Bull, 1985; 15(2):221–223.
7. Jones BE, Bobillier P, Pin C, Jouvet M. The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking in the cat. Brain Res, 1973; 58(1):157–177.
8. Lu J, Xu M, Saper C. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray (PAG). Jour Neurosci 26(1):193–202.
9. Gainetdinov RR, Caron MG. Monoamine transporters: from genes to behavior. Annu Rev Pharmacol Toxicol, 2003; 43:261–284.
10. Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D. Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. J Neurosci, 2001; 21(16):5916–5924.
11. Davids E, Zhangf K, Kula NS, Tarazi FI, Baldessarini RJ. Effects of norepinephrine and serotonin transporter inhibitors on hyperactivity induced by neonatal 6-hydroxydopamine lesioning in rats. J Pharmacol Exp Ther, 2002; 301(3):1097–1102.
12. Fischer JF, Cho AK. Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. J Pharmacol Exp Ther, 1979; 208(2):203–209.
13. Saunders C, Ferre JV, Shi L, Chen J, Merril G, Lamb ME, Leeb-Lundberg LM, Carvelli L, Javitch JA, Galli A, Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. Proc Natl Acad Sci U S A, 2000; 97(12):6850–6855.

14. Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci, 1998; 18(6):1979–1986.
15. Brown JM., Hanson GR, Fleckenstein AE, Methamphetamine rapidly decreases vesicular dopamine uptake. J Neurochem, 2000; 74(5):2221–2223.
16. Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR., VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. Proc Natl Acad Sci U S A, 1997; 94(18):9938–9943.
17. Fon EA, Pothos EN, Sun BC, Killeen N, Sulzer D, Edwards RH. Vesicular transport regulates monoamine storage and release but is not essential for amphetamine action. Neuron, 1997; 19(6):1271–1283.
18. Gonzalez AM, Walther D, Pazos A, Uhl GR. Synaptic vesicular monoamine transporter expression: distribution and pharmacologic profile. Brain Res Mol Brain Res, 1994; 22(1–4):219–226.
19. Sulzer D, Rayport S. Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. Neuron, 1990; 5(6):797–808.
20. Mantle TJ.,Tipton KF, Garrett NJ. Inhibition of monoamine oxidase by amphetamine and related compounds. Biochem Pharmacol, 1976; 25(18):2073–2077.
21. Nishino S, Mao J, Sampathkumaran R, Shelton J, Mignot E Increased dopaminergic transmission mediates the wake-promoting effects of CNS stimulants. Sleep Res Online, 1998; 1(1):49–61.
22. Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM Dopaminergic role in stimulant-induced wakefulness. J Neurosci, 2001; 21(5):1787–1794.
23. Jones BE.,Harper ST, Halaris AE, Effects of locus coeruleus lesions upon cerebral monoamine content, sleep-wakefulness states and the response to amphetamine in the cat. Brain Res, 1977; 124(3):473–496.
24. Xu F, Gainetdinov RR, Wetsel WC, Jones SR, Bohn LM, Miller GW, Wang YM, Caron MG, Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. Nat Neurosci, 2000; 3(5):465–471.
25. Duteil J, Rambert FA, Pessonnier J, Hermant JF, Gombert R, Assous E., Central alpha 1-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil; studies with experimental animals. Eur J Pharmacol, 1990; 180(1):49–58.
26. Lin JS, Roussel B, Akaoka H, Fort P, Debilly G, Jouvet M. Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. Brain Res, 1992; 591(2):319–326.
27. Mignot E, Nishino S, Guillemeinault C, Dement WC. Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep, 1994; 17(5):436–437.
28. Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, Tassin JP Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci, 2002; 22(7):2873–2884.
29. Shelton J, Nishino S, Vaught J, Dement WC, Mignot E., Comparative effects of modafinil and amphetamine on daytime sleepiness and cataplexy of narcoleptic dogs. Sleep, 1995; 18(10):817–826.
30. Edgar DM, Seidel WF, Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. J Pharmacol Exp Ther, 1997; 283(2):757–769.
31. Saletu, B, Saletu M, Grunberger J, Frey R, Anderer P, Mader R. Treatment of the alcoholic organic brain syndrome: double-blind, placebo-controlled clinical, psychometric and electroencephalographic mapping studies with modafinil. Neuropsychobiology, 1993; 27(1):26–39.
32. Jasinski DR. An evaluation of the abuse potential of modafinil using methylphenidate as a reference. in Congress on Problems in Drug Dependance. 1997. Nashville.
33. Buguet A, Montmayeur A, Pigeau R, Naitoh P. Modafinil, d-amphetamine and placebo during 64 hours of sustained mental work. II. Effects of two nights of recovery sleep. J. Sleep Res., 1995; 4:229–241.
34. Wong, YN Wang L, Hartman L, Simcoe D, Chen Y, Laughton W, Eldon R, Markland C, Grebow P. Comparison of the single-dose pharmacokinetics and tolerability of modafinil and dextroamphetamine administered alone or in combination in healthy male volunteers. J Clin Pharmacol, 1998; 38(10):971–978.
35. Hermant JF, Rambert FA, Duteil J. Awakening properties of modafinil: effect on nocturnal activity in monkeys (Macaca mulatta) after acute and repeated administration. Psychopharmacology (Berl), 1991; 103(1):28–32.
36. Rush, CR, Kelly TH, Hays LR, Baker RW, Wooten AF. Acute behavioral and physiological effects of modafinil in drug abusers. Behav Pharmacol, 2002; 13(2):105–115.

37. Makris AP, Rush CR, Frederich RC, Kelly TH. Wake-promoting agents with different mechanisms of action: comparison of effects of modafinil and amphetamine on food intake and cardiovascular activity. Appetite, 2004; 42(2):185–195.
38. Wong YN, Wang L, Hartman,Simcoe D, Chen Y, Laughton W, Eldon R, Markland C, Grebow P.., A double-blind, placebo-controlled, ascending-dose evaluation of the pharmacokinetics and tolerability of modafinil tablets in healthy male volunteers. J Clin Pharmacol, 1999; 39(1):30–40.
39. Billiard M, Besset A, Montplaisir J, Laffont F, Goldenberg F, WeillJS, Lubin S. Modafinil: a double-blind multicentric study. Sleep, 1994; 17(8 Suppl):S107–S112.
40. Broughton RJ, Fleming JAE, George CFP, Hill JD, Kryger MH, Moldofsky H, Montplaisir J, Morehouse RL, Moskovitch A, Murphy WF. Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. Neurology, 1997; 49(2):444–451.
41. Saletu B, Saletu M, Grunberger J, Frey R, Zatschek I, Mader., On the treatment of the alcoholic organic brain syndrome with an alpha-adrenergic agonist modafinil: double-blind, placebo-controlled clinical, psychometric and neurophysiological studies. Prog Neuropsychopharmacol Biol Psychiatry, 1990; 14(2):195–214.
42. Moldofsky H., Broughton RJ, Hill JD. A randomized trial of the long-term, continued efficacy and safety of modafinil in narcolepsy. 2000; 1(2):109–116.
43. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology, 2000; 54(5):1166–1175.
44. Sackner-Bernstein J,. Niebler G and C. Earl C. Effect of the Centrally Acting Wake-Promoting Agent Modafinil on Blood Presure and heart Rate. SLEEP, 2003; 26:A80.
45. Tanganelli S, Fuxe K, Ferraro L, Janson AM, Bianchi C. Inhibitory effects of the psychoactive drug modafinil on gamma- aminobutyric acid outflow from the cerebral cortex of the awake freely moving guinea-pig. Possible involvement of 5-hydroxytryptamine mechanisms. Naunyn Schmiedebergs Arch Pharmacol, 1992; 345(4):461–465.
46. Tanganelli S, Perez de la Mora, Ferraro L, Mendez-Franco J, Beani L, Rambert FA, Fuxe K. Modafinil and cortical gamma-aminobutyric acid outflow. Modulation by 5-hydroxytryptamine neurotoxins. Eur J Pharmacol, 1995; 273(1–2):63–71.
47. Ferraro L, Tanganelli S, OConnor WT, Antonelli T, Rambert FA, Fuxe K. The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. Eur J Pharmacol, 1996; 306(1–3):33–39.
48. Ferraro L, Antonelli T, Tanganelli S, OConnor WT, Perez de la Mora M, Mendez-Franco J, Rambert FA. The vigilance promoting drug modafinil increases extracellular glutamate levels in the medial preoptic area and the posterior hypothalamus of the conscious rat: prevention by local GABAA receptor blockade. Neuropsychopharmacology, 1999; 20(4):346–356.
49. Perez de la Mora, M., Aguilar-Garcia A, Ramon-Frias T,Ramirez-Ramirez R, Mendez-Franco J, Rambert F, Fuxe K., Effects of the vigilance promoting drug modafinil on the synthesis of GABA and glutamate in slices of rat hypothalamus. Neurosci Lett, 1999; 259(3):181–185.
50. Tanganelli S, Ferraro L, Bianchi C, Fuxe K. 6-hydroxy-dopamine treatment counteracts the reduction of cortical GABA release produced by the vigilance promoting drug modafinil in the awake freely moving guinea-pig. Neurosci Lett, 1994; 171(1–2):201–204.
51. Ferraro L, Tanganelli S, OConnor WT, Antonelli T, Rambert F, Fuxe K. The vigilance promoting drug modafinil decreases GABA release in the medial preoptic area and in the posterior hypothalamus of the awake rat: possible involvement of the serotonergic 5-HT3 receptor. Neurosci Lett, 1996; 220(1):5–8.
52. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammel TE, Lee C, Richardson JA, Williams SC, Xiong Y, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell, 1999; 98(4):437–451.
53. Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Miller MS, Saper CB Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci, 2000; 20(22):8620–8628.
54. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol, 1998; 43(1):88–97.
55. Willie J, Renthal W, Chemelli RM, Miller MS, Scammel TE, Yanagisawa M, Sinton CML.Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience, 2005; 130: 983–995.

56. Lin JS, Hou Y, Jouvet M. Potential brain neuronal targets for amphetamine-, methylphenidate-, and modafinil-induced wakefulness, evidenced by c-fos immunocytochemistry in the cat. Proc Natl Acad Sci U S A, 1996; 93(24):14128–14133.
57. Engber TM, Koury EJ, Dennis SA, Miller MS, Contreras PC, Bhat RV. Differential patterns of regional c-Fos induction in the rat brain by amphetamine and the novel wakefulness-promoting agent modafinil. Neurosci Lett, 1998; 241(2–3):95–98.
58. Ko EM, Estabrooke IV, McCarthy M, Scammell TE. Wake-related activity of tuberomammillary neurons in rats. Brain Res, 2003; 992(2):220–226.
59. Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammel TE. Fos expression in orexin neurons varies with behavioral state. J Neurosci, 2001; 21(5):1656–1662.
60. LaHoste GJ, Yu J, Marshall JF. Striatal Fos expression is indicative of dopamine D1/D2 synergism and receptor supersensitivity. Proc Natl Acad Sci U S A, 1993; 90(16):7451–7455.
61. Cole AJ, Bhat RV, Patt C, Worley PF, Baraban JM. D1 dopamine receptor activation of multiple transcription factor genes in rat striatum. J Neurochem, 1992; 58(4):1420–1426.
62. de Saint Hilaire, Z., Orosco M, Rouch C, Blanc G, Nicolaidis S., Variations in extracellular monoamines in the prefrontal cortex and medial hypothalamus after modafinil administration: a microdialysis study in rats. Neuroreport, 2001; 12(16):3533–3537.
63. Fuxe K, Janson AM, Rosen, Finnman UB, Tanganelli S, Moran M, Goldstein M, Agnati LF. Evidence for a protective action of the vigilance promoting drug modafinil on the MPTP-induced degeneration of the nigrostriatal dopamine neurons in the black mouse: an immunocytochemical and biochemical analysis. Exp Brain Res, 1992; 88(1):117–130.
64. Moachon G, Kanmacher I, Clenet M, Matinier D. Pharmacokinetic profile of modafinil. Drugs of Today, 1996; 32(4):327–337.
65. Gallopin T, Luppi PH, Rambert FA, Frydman A, Fort P.. Effect of the Wake-Promoting Agent Modafinil on Sleep-Promoting Neurons from the Ventrolateral Preoptic Nucleus: an In Vitro Pharmacologic Study. Sleep, 2004; 27(1):19–25.
66. Jones SR, Gainetdinov RR, Hu XT, Cooper DC, Wightman RM, White FJ, Caron MG. Loss of autoreceptor functions in mice lacking the dopamine transporter. Nat Neurosci, 1999; 2(7):649–655.
67. Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M. Identification of sleep-promoting neurons in vitro. Nature, 2000; 404(6781):992–995.
68. Akaoka H, Roussel B, Lin JS, Chouvet G, Jouvet M. Effect of modafinil and amphetamine on the rat catecholaminergic neuron activity. Neurosci Lett, 1991; 123(1):20–22.
69. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol, 1997; 52(1):27–78.
70. Ferraro L, Fuxe K, Tanganelli S, Fernandez M, Rambert FA, Antonelli T. Amplification of cortical serotonin release: a further neurochemicaal action of the vigilance-promoting drug modafinil. Neuropharmacology, 2000; 39(11):1974–1983.
71. Ferraro L, Fuxe K, Tanganelli S, Tomasini MC, Rambert FA, Antonelli T. Differential enhancement of dialysate serotonin levels in distinct brain regions of the awake rat by modafinil: possible relevance for wakefulness and depression. J Neurosci Res, 2002; 68(1):107–112.
72. Ishizuka T, Sakamoto Y, Sakurai T, Yamatodani A. Modafinil increases histamine release in the anterior hypothalamus of rats. Neurosci Lett, 2003; 339(2):143–146.
73. Simon P, Hemet C, Ramassamy C, Costentin J. Non-amphetaminic mechanism of stimulant locomotor effect of modafinil in mice. Eur Neuropsychopharmacol, 1995; 5(4):509–514.
74. Saletu, B, Frey r, Krupka M, Anderer P, Grunberger J, Barbaroj MJ, Differential effects of a new central adrenergic agonist-modafinil- and D-amphetamine on sleep and early morning behaviour in young healthy volunteers. Int J Clin Pharmacol Res, 1989; 9(3):183–195.
75. Saletu B, Frey R, Krupka M, Anderer P, Grunberger J, Barbanoj MJ. Differential effects of the new central adrenergic agonist modafinil and d-amphetamine on sleep and early morning behaviour in elderlies. Arzneimittelforschung, 1989; 39(10):1268–1273.
76. Batejat DM, Lagarde DP. Naps and modafinil as countermeasures for the effects of sleep deprivation on cognitive performance. Aviat Space Environ Med, 1999; 70(5):493–498.

51
Modafinil: The European Experience

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

AGNES NICOLET
School of Pharmacy, Montpellier, France

YVES DAUVILLIERS
CHU Montpellier, Unité de Sommeil Hôpital Gui de Chauliac, Montpellier, France

BERTRAND CARLANDER
Department of Neurology, Gui de Chauliac Hospital, Montpellier, France

The European experience on modafinil includes chemical, pharmacological, and clinical studies. Among the later are, studies on sleep (1–2), hormones (3) and cognitive functions (4–5) in healthy volunteers; alcoholic organic brain syndrome (6–7); cognitive performance during sleep deprivation experiments (8–9) and sustained military operations (10–11); residual excessive daytime sleepiness in the sleep apnea/hypopnea syndrome (12–13); fatigue in multiple sclerosis (14–15), excessive daytime sleepiness in Parkinson's disease (16–17) and above all studies directed more closely towards narcolepsy, which will be developed in this chapter. All these studies have been concentrated in 3 countries, France, Austria, and the United Kingdom, in connection with Lafon Ltd, a company based in Maisons-Alfort (France), purchased by the U.S. company Cephalon in 2000. In this chapter we will concentrate on five aspects: the discovery of modafinil and the first trials in animals and humans; the first open-label study and the first multicenter, randomized, placebo-controlled trial of Modafinil in the treatment of excessive daytime sleepiness in narcolepsy; the *c-fos* immunocytochemical study carried out in the cat to identify the potential target neurons of modafinil and compare them with those for amphetamine and methylphenidate; the effect of the functional polymorphism of the catechol-O-methyltransferase (COMT) gene in the response to modafinil and the world longest open-label study (up to 20 years) in narcoleptic subjects treated with modafinil.

I. Discovery and Development of Modafinil

The history of modafinil dates back to 1974. As they were screening molecules in search of analgesics, two chemists from Lafon Ltd, Assous and Gombert, identified a new molecule, adrafinil [(diphenylmethyl) sulfinyl-2 acetohydroxamic acid] (Fig. 1), later passed over to two pharmacologists, also from Lafon Ltd., Duteil and Rambert, for pharmacological screening. The latter observed that mice treated with this molecule were hyperactive and concluded to its potential interest (18–19). Following further tests in mice and

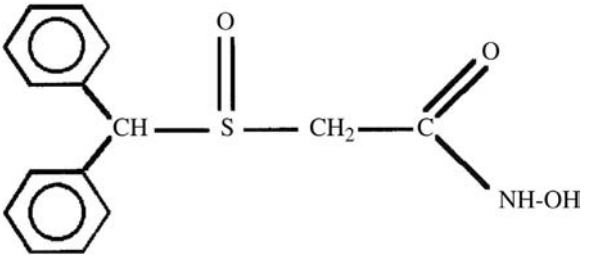


Figure 1 Chemical structure of adrafinil. Adrafinil is a 2-[(diphenylmethyl) sulfinyl] acetohydroxamic acid with a main inactive glucuro-conjugate metabolite and a secondary active metabolite, modafinil.

rats, and more refined pharmacology in dogs, the molecule was passed over to Jouvet for evaluation in the cat and to Milhaud and Klein for evaluation in the monkey. An increase of electroencephalographic wakefulness was found by the first group, and an increase of nocturnal activity by the second one. Eventually, in 1977–1978, Jouvet prescribed adrafinil to narcoleptic subjects with inconstant results (Table 1).

Meanwhile, in 1976, the kinetic of adrafinil led to the identification of an active metabolite, modafinil [(diphenylmethyl) sulfinyl-2 acetamide] (Fig. 2). Modafinil went through the same steps of development leading to the demonstration of a dose-dependent increase in locomotor activity in mice (20), an increase of electroencephalographic wakefulness in the cat (21), an increase of electroencephalographic wakefulness (22) and an increase in nocturnal activity and in behavioral arousal without stereotyped behavior (23) in rhesus monkeys. As soon as early 1983 Jouvet prescribed modafinil to narcoleptic and idiopathic hypersomnia subjects. The results overdid the expectations. Jouvet's first patient, a farmer whose fields were by the side of a road, was regularly finding himself driving his tractor across the road in a state of drowsiness or asleep. From one day to the other the farmer became alert and secure on the road. From then

Table 1 An Early History of Modafinil

1974: Identification of a new molecule, *adrafinil*, at Lafon Ltd (Maisons-Alfort, France), responsible for:
- a hyperactivity in the mouse (Duteil and Rambert, 1979),
- an increase of EEG wakefulness in the cat (Jouvet, unpublished)
- and an increase of nocturnal activity in the rhesus monkey (Milhaud and Klein, unpublished)

1977–1978: Adrafinil is prescribed to narcoleptic subjects with inconstant results (Jouvet, unpublished)

1976: The kinetic of adrafinil leads to an active metabolite, referred to as *modafinil*, responsible for:
- a dose-dependent increase of locomotor activity in the mouse (Duteil et al. 1990)
- an increase of EEG wakefulness in the cat (Lin et al. 1992)
- and an increase in nocturnal activity in the rhesus monkey (Lagarde and Milhaud, 1990)

1983: Modafinil is prescribed to narcoleptic subjects with great success (Bastujji and Jouvet, 1988)

1984: Start of clinical trials in both normal and narcoleptic subjects, by Lafon Ltd

1992: Official registration of modafinil in France

1994: Publication of the first multicenter, randomized, placebo-controlled trial of modafinil (Billiard et al. 1994) and commercial availability of modafinil in France

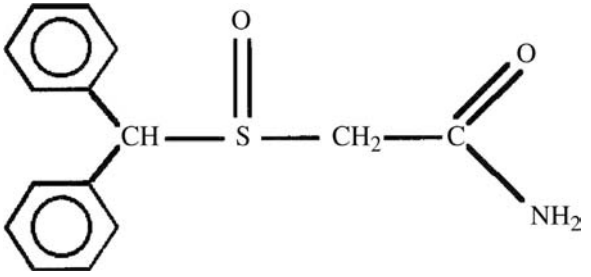


Figure 2 Chemical structure of modafinil. Modafinil is a 2-[(diphenylmethyl) sulphinyl] acetamide (the NH-OH radical of adrafinil is replaced by NH_2).

on Jouvet continued to use modafinil with success in both narcoleptic and idiopathic hypersomnia subjects. In 1984 Lafon Ltd decided to start clinical trials in both healthy volunteers and narcoleptics under the leadership of Weil and Lubin, clinical directors at Lafon Ltd. First studies of the effects of modafinil on night sleep and daytime sleepiness in healthy volunteers were conducted by Goldenberg et al. (1) and Saletu et al (2). The first one revealed decreased total sleep time, decreased NREM sleep stages 3 and 4, no modification of REM sleep and no rebound phenomenon after a single evening dose of modafinil 200 mg or placebo in parallel groups. In addition, sleep latency on the multiple sleep latency test (MSLT) increased in every single session following a single dose of modafinil, 200 mg at 10:00 AM. The second one compared the effects of modafinil 100 and 200 mg, *d*-amphetamine 10 and 20 mg and placebo, on night sleep. A decreased total sleep time and sleep efficiency was observed with modafinil 200 mg, much less marked than with amphetamines. These and other studies in healthy volunteers were accompanied by open-label trials in subjects with narcolepsy or idiopathic hypersomnia performed in different centers and by a multicenter, randomized, placebo-controlled study, leading eventually to the official registration of modafinil in France in June 1992 and his commercial availability also in France, in September 1994.

II. First Clinical Publications

The first publication on modafinil, *Successful treatment of idiopathic hypersomnia and narcolepsy* (24), was the outcome of an open label study in which the drug was given to 24 narcoleptic and 18 idiopathic hypersomnia subjects. The drug was administered in the morning and at noon, at a dose of 200 to 500 mg, according to the patient's weight and the severity of the symptom. An improvement of excessive daytime sleepiness was obtained in 71% of narcoleptic and in 83% of idiopathic hypersomnia subjects, while the drug was ineffective in 17% of narcoleptic and 6% of idiopathic hypersomnia subjects only. In addition, this study reported on a massive dose of the drug (45 tablets of 100 mg) ingested by a 21-year-old female, as an attempt to suicide, without serious deleterious effect.

The first multicenter, randomized, placebo controlled trial of modafinil came out later (25). Fifty narcoleptic subjects (33 men and 17 women) were selected in four different centers (Montpellier, Montreal, Paris and Creteil) according to the following criteria: presence of excessive daytime sleepiness and cataplexy, a mean sleep latency of less than seven minutes and two or more sleep onset REM periods on the MSLT and

an association with HLA DR2-DQ1. Subjects were either free of drugs or had discontinued psychostimulant medication for at least 14 days before entering the study. Anticataplectic drugs were continued in a few subjects with severe cataplexy. Modafinil was administered in a double-blind cross-over design, at a dosage of 300 mg versus placebo. The duration of the study was 12 weeks, including a 2-week « run in » period during which subjects received placebo, a first 4-week treatment period with either modafinil or placebo, a 2-week wash out period with placebo, and a second 4-week treatment period with either placebo or modafinil. At the beginning and at the end of each treatment period subjects spent 24 hours in the sleep laboratory for sleep evaluation (22:30 – 07:30) and a maintenance of wakefulness test the following day. Throughout the study subjects filled out a sleep log at home. Polysomnographic nights were preceded by a questionnaire on therapeutic effects judged both by a physician and by the patient, and followed by a sleep questionnaire. Sleep logs did not show any modification of night sleep, but a reduction of daytime sleepiness and overwhelming episodes of sleep. Cataplexy was not modified. Sleep questionnaires did not disclose any significant modification of sleep continuity and quality. An overall clinical benefit was noted by physicians as well as by subjects. Above all there was a significant improvement in the results of the MWT for patients on modafinil in comparison with placebo ($p < 0.05$).

III. Potential Brain Arousal Targets for Amphetamine, Methylphenidate, and Modafinil-Induced Wakefulness, Evidenced by c-*fos* Immunocytochemistry in the Cat and the Rat

In an effort to identify the brain targets of modafinil and amphetamine in the central nervous system, Lin et al. (26) developed *c-fos* immunocytochemical procedures in cats. Animals were sacrificed 60 min after a single oral administration of amphetamine, methylphenidate, or modafinil at equivalent doses for wake induction (1, 2.5, or 5 mg/kg, respectively) and brain sections examined for Fos by immunocytochemistry. Interestingly, both methylphenidate and amphetamine evoked a widespread and dense *c-fos* expression in a large number of brain areas such as in the striatum and whole cortex, especially in the caudate nucleus and mediofrontal cortex known to be dopamine targets. In contrast modafinil elicited no or few labelled cells in striatal and cortical regions, but did induce marked *c-fos* labelling in neurons of the anterior hypothalamic nucleus, as well as in other localized areas such as the pontine and periaqueductal gray. In view of the small size of the anterior hypothalamic nucleus and the large number and high intensity of the *c-fos* labelling on it, the authors considered this nucleus as the major structure in which modafinil induces *c-fos* expression. In a more recent study (27), following the discovery of the involvement of the hypocretin (orexine) system in narcolepsy, *c-fos* immunocytochemical procedures was used in rats. In this later study rats were sacrificed 120 min after an ip 75–150 mg/kg dose. An increased Fos immunoreactivity was found in the tubero-mamillary nucleus (TMN) and in the hypocretin neurons of the perifornical area, two cell groups involved in the regulation of wakefulness, not in either the suprachiasmatic nucleus or the anterior hypothalamic area. The discrepancy between the results of the two studies may include species differences, dose used, and delay in sacrifice.

IV. Functional Polymorphism of the COMT Gene as a Critical Factor in the Response to Modafinil

It is commonly observed that drug response varies considerably between individuals. Differences in drug response depend on several factors such as body mass index, age gender, and genetics. In the case of modafinil the gene for COMT seemed of special interest given its key modulatory role in the dopamine and noradrenaline neurotransmission and the fact that modafinil might act through the dopamine system (28). Accordingly, a functional polymorphism of COMT was analyzed in 97 narcoleptic subjects, 59 men and 38 women, affected with typical narcolepsy with cataplexy (29). This analysis led to the finding of a sexual dimorphism and a strong effect of COMT genotype on disease severity. In addition 84 out of the previously reported narcoleptic subjects, 52 men and 32 women, were analyzed for the effect of modafinil, based on clinical evaluation (30). 52 of these subjects were good responders. The gender as such did not affect the response to modafinil whereas a strong effect of COMT genotype was found on response to modafinil, with a better response in the subjects with a low COMT enzyme activity genotype (high dopamine metabolizers) than in subjects with a high COMT enzyme activity genotype (low dopamine metabolizers) (Fig. 3). Thus the usual dimorphism found in the COMT gene in narcolepsy was extended to the response to modafinil, with the optimal daily dose of modafinil being almost 100 mg per day less in women than in men.

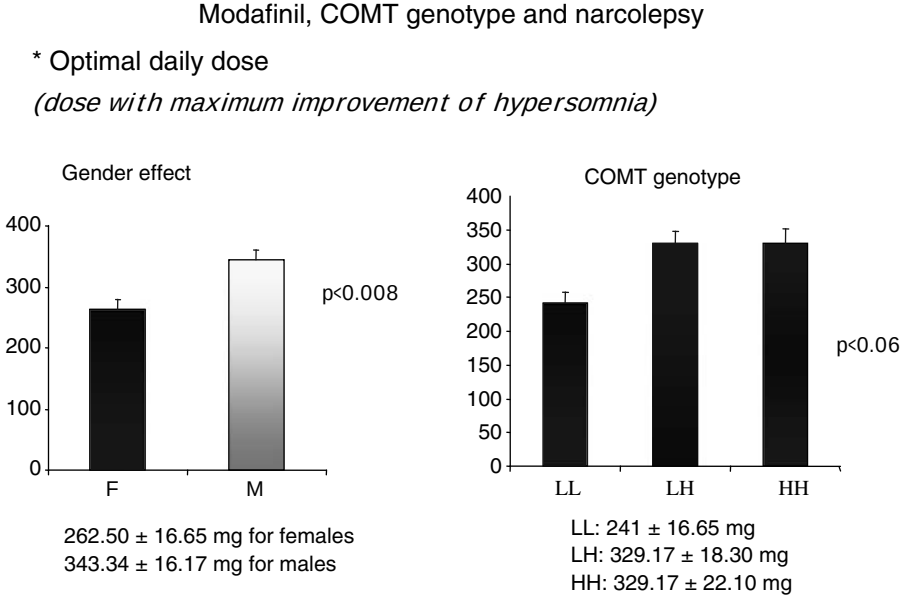


Figure 3 Differences in daily dose between gender and COMT genotypes: the optimal daily dose of modafinil is significantly lower in women than in men, and slightly lower in subjects with the LL genotype than in subjects with the LH or HH genotype.

V. Long-Term Efficacy and Safety of Modafinil for the Treatment of Excessive Daytime Sleepiness in Narcoleptic Subjects

This study was conducted in a population of 244 narcolepsy with cataplexy subjects, 160 men and 84 women, referred to the Montpellier University Sleep and Wake Disorders Center from April 1984 to July 1998, and followed-up until August 2004.

Median age of onset of excessive daytime sleepiness was 19.5 years (2–50) and median age of onset of cataplexy 27.0 years (6–56). 188 subjects (77%) had a sporadic form of narcolepsy while 56 (22.9%) had a familial form, including 16 subjects (6.5%) with one or more relatives affected with narcolepsy with cataplexy and 40 subjects (16.3%) with one or more relatives affected with isolated recurrent naps and/or lapses into sleep only. Modafinil was the first treatment in 139 subjects (56.9%), while it had been preceded by another stimulant in the past in 65 subjects (26.6%), and just before the introduction of modafinil in 40 subjects (16.3%)

The severity of the condition was evaluated on the Epworth sleepiness scale, on the frequency of cataplexy on a scale from 0 (less than one cataplexy per year) to 5 (several attacks per day), on nighttime polysomnographic parameters, and on the mean sleep latency and number of sleep onset REM periods (SOREMPs) on the MSLT. Modafinil was started at a daily dose of 200 mg and subjects were asked to come back for a first follow-up visit one month later. The daily dose was then adjusted according to clinical results. Subsequently patients returned to the outpatient clinic at their own will and modafinil was further adjusted if necessary. Efficacy was judged by the attending physician and graded as 2 (effective or very effective), 1 (partially effective) and 0 (ineffective).

The best result of modafinil on EDS was graded 2 in 137 subjects (56.1%), 1 in 49 (20.0%) and 0 in 40 subjects (16.4%). 18 subjects (7.3%) dropped out after the first prescription so that the effects of treatment could not be assessed (Fig. 4). In addition, the effectiveness of the starting dose (200 mg) was tested in 156 subjects using survival analysis (Kaplan Meier's method). A significantly better response to modafinil

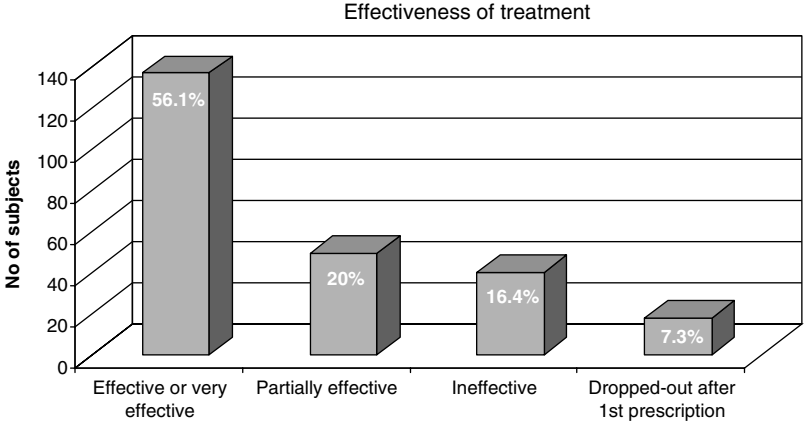


Figure 4 Effectiveness of treatment with modafinil in 244 narcolepsy with cataplexy subjects started on modafinil between April 1984 and July 1998.

Table 2 Long-Term Follow-Up of 244 Narcolepsy with Cataplexy Subjects Started on Modafinil Between April 1984 and July 1998

Outcome	Number of subjects	%	Duration (median)	Range
Still on treatment	97	39.7	8 yr	1 / 20 yr
Discontinuation of treatment			Delay since onset of treatment (median)	
No response at all	24	9.8	2 mon	7 days / 4 mon
Insufficient response	17	6.9	18 mon	6 mon / 6 yr
Response fading out	38	15.5	15 mon	1 mon / 10 yr
Adverse effect	20	8.1	5.5 mon	<1 mon / 5 yr
Other reason	14	5.7	32 mon	1 mon / 8 yr
Pattern of discontinuation/ and resumption of treatment	6	2.4		
Lost contact with, after first prescription within the first year of treatment	18 10	7.3 4.1		

(log rank test) was found in patients with total sleep time >7 hours during polysomnographic recording ($p = 0.0328$), in patients under 25 years of age ($p = 0.0366$), in patients with no previous use of stimulants ($p = 0.0407$) and in patients with a sporadic form versus a familial form ($p = 0.0430$)

Results of long-term follow-up of the subjects are displayed in Table 2. 39.7% of the subjects were still on treatment at the time of their last visit, including 10 subjects for 15 years or more, while 46.0% had discontinued the drug for various reasons. 2.4% showed a pattern of discontinuation and resumption of treatment, and 11.4% were lost contact with, either after the first visit or within the first year of treatment, a number of subjects in this last category originating from foreign countries or remote French territories. Most interestingly, discontinuation of modafinil for any reason was exceptional after 5 years of treatment. These results are of paramount importance in documenting a lack of dependence, and almost certainly of tolerance with sustained use of modafinil.

VI. Conclusion

Early studies on modafinil tend to be forgotten. However it would be fair enough to acknowledge the pioneering work by Lafon Ltd., and the past and present active contributions of various European groups, both in the field of narcolepsy and other fields such as the effects of modafinil in sleep deprived subjects, in the residual excessive daytime sleepiness of obstructive sleep apnea/hypopnea subjects treated by CPAP, in fatigue of multiple sclerosis and excessive daytime sleepiness of Parkinson's disease.

References

1. Goldenberg F, Weil JS, Von Frenckeel. Effects of modafinil on diurnal variation of objective sleepiness in normal subjects. Sleep Res 1987; 16:91.
2. Saletu B, Frey R, Krupka M, Anderer P, Grunberger J, Barbanoj MJ. Differential effects of a new central adrenergic agonist modafinil and d-amphetamine on sleep and early morning behaviour in young healthy volunteers. Int J Clin Pharm Res 1989; 9:183–195.

3. Brun J, Chamba G, Khalfallah Y, Girard P, Boissy I, Bastuji, Sassolas G, Claustrat B. Effects of modafinil on plasm melatonin, cortisol and growth hormone rhythms, rectal temperature and performance in healthy subjects during a 36 hour sleep deprivation. J Sleep Res 1998; 7:105–114
4. Turner DC, Robbins TW, Clark L, Aron AR, Dowson J, Sahakian BJ. Cognitive enhancing effects of modafinil in healthy volunteers. Psychopharmacology (Berl) 2003; 165:260–269.
5. Randall DC, Shneerson JM, Plaha KK, File SE. Modafinil affects mood, but not cognitive function, in healthy young volunteers. Hum Psychopharmacol 2003; 18:163–173.
6. Saletu B, Saletu M, Grunberger J, Frey R, Zatschek I, Mader R. On the treatment of the alcoholic organic brain syndrome with an alpha-adrenergic agonist modafinil: double-blind, placebo-controlled clinical, psychometric and neurophysiological studies. Prog Neuropsychopharmacol Biol Psychiatry 1990; 14:195–214.
7. Saletu B, Saletu M, Grunberger J, Frey R, Andere P, Mader R. Treatment of the alcoholic organic brain syndrome: double-blind, placebo-controlled clinical, psychometric and electroencephalographic mapping studies with modafinil. Neuropsychobiology 1993; 27:26–39.
8. Lagarde D, Batejat D, Van Beers P, Sarafian D, Pradella S.. Interest of modafinil, a new psychostimulant, during a sixty-hour sleep deprivation experiment. Fundam Clin Pharmacol 1995; 9:271–279.
9. Batejat DM, Lagarde DP. Naps and modafinil as countermeasures for the effects of sleep deprivation on cognitive performance. Aviat Space Environ Med 1999; 70:493–498.
10. Buguet A, Montmayeur A, Pigeau R, Naitoh P. Modafinil, *d*-amphetamine and placebo during 64-hours of sustained mental work. II. Effects on two nights of recovery sleep.
11. Buguet A, Moroz DE, Radomski MW. Modafinil-medical considerations for use in sustained operations. Aviat Space Environ Med 2003; 74:659–663.
12. Arnulf I, Homeyer P, Garma L, Whitelaw WA, Derenne JP. Modafinil in obstructive sleep apnea-hypopnea syndrome: a pilot study in 6 patients. Respiration, 1997; 64:159–161.
13. Kingshott RN, Vennelle M, Coleman EL, Engleman HM, Mackay TW, Douglas NJ. Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of residual excessive daytime sleepiness in the sleep apnea/hypopnea syndrome. Am J Respir Crit Care Med 2001; 163:918–923.
14. Zifko UA, Rupp M, Schwarz S, Zipko HT, Maida EM. Modafinil in treatment of fatigue in multiple sclerosis. Results of an open-label study. J Neurol 2002; 249:983–987.
15. Zifko UA. Management of fatigue in patients with multiple sclerosis. Drugs 2004; 64:1295–1304.
16. Montastruc JL, Rascol O. Modafinil and pramipexole-associated somnolence. Mov Disord 2001; 16:783–784.
17. Hogl B, Saletu M, Brandauer E, Glatzl S, Frauscher B, Seppi K, Ulmer H, Wenning G, Poewe W. Modafinil for the treatment of daytime sleepiness in Parkinson's disease: a double-blind, randomized, crossover, placebo-controlled polygraphic trial. Sleep 2002; 25:905–909.
18. Duteil J, Rambert FA, Pessonnier J, Gombert R, Assous E. A possible α-adrenergic mechanism for drug (CRL 40028) – induced hyperactivity. Eur J Pharmacol 1979; 121–123.
19. Rambert FA, Pessonnier J, de Sereville JE, Pointeau AM, Duteil J. Profil psychopharmacologique original de l'adrafinil chez la souris. J Pharmacol (Paris) 1986; 17:37–52.
20. Duteil J, Rambert FA., Pessonnier J., Hermant JF, Gombert R, Assous E.. Central α-1-adrenergic stimulation in relation to the behaviour stimulating effect of modafinil: studies with experimental animals. Eur J Pharmacol 1990; 180:49–58.
21. Lin J, Roussel B, Akaoka H, Fort P, Debilly G, Jouvet M. Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. Brain Res 1992; 591:19–26.
22. Lagarde D, Milhaud C. Electroencephalographic effects of modafinil, an α-1- adrenergic psychostimulant, on the sleep of rhesus monkey. Sleep 1990; 13:441–448.
23. Hermant JF, Rambert FA, Duteil J. Awakening properties of modafinil: effect on nocturnal acticity in monkeys (*Macaca mulatta)* after acute and repeated administration. Psychopharmacology 1991; 103:28–32.
24. Bastujji H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuro-Psychopharmacol & Biol Psychiat 1988; 12:695–700.
25. Billiard M, Besset A, Montplaisir J, Laffont F, Goldenberg F, Weill JS, Lubin S. Modafinil: a double blind multicentric study. Sleep 1994; 17:S107–S112.

26. Lin JS, Hou Y, Jouvet M. Potential brain neuronal targets for amphetamine-, methyphenidate-, and modafinil-induced wakefulness, evidenced by c-*fos* immunocytochemistry in the cat. Proc Natl Acad Sci USA. 1996; 93:14128–14133.
27. Scammell TE, Estabrooke IV, McCarthy MT, Chemelli RM, Yanagisawa M, Saper CB, Miller MS, Saper CB. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000; 20:8620–8628.
28. Mignot E, Nishino S, Guilleminault C, Dement WC. Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep 1994; 17:436–437.
29. Dauvilliers Y, Neidhart E, Lecendreux M., Billiard M, Tafti M. MAO-A and COMT polymorphisms and gene effects in narcolepsy. Mol Psychiatry 2001; 6:367–372.
30. Dauvilliers Y, Neidhart E, Billiard M, Tafti M. Sexual dimorphism of the catecho-O-methyltransferase gene in narcolepsy is associated with response to modafinil. The Pharmacogenomics Journal 2002; 2:65–68

52
Modafinil: The U.S. Experience

MICHAEL THORPY
Department of Neurology, Sleep-Wake Disorders Center, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, U.S.A.

I. The Introduction of Modafinil in the United States

Clinical research in the United States on the wake-promoting agent modafinil began in 1993, when Cephalon, Inc., a Pennsylvania-based biotechnology firm, licensed the rights to modafinil in the U.S. from its developer, French pharmaceutical company Laboratorie L. Lafon (1). Modafinil was approved for use in narcolepsy in France in 1992, and had been studied in other disorders of excessive sleepiness in that country, as well as for depression and "vigilance enhancement" (1,2).

Cephalon received orphan drug status for modafinil in 1993 under the Federal Food, Drug, and Cosmetic Act, which provides tax incentives and 7-year marketing exclusivity for companies that sponsor treatments for rare diseases (3). Cephalon conducted preclinical and phase I studies on pharmacokinetics and mechanism of action of modafinil (4,5,6) that supplemented previous French research in these areas. Studies in the U.S. were also initiated to the compare the abuse liability of modafinil with that of central nervous system (CNS) stimulants. While the mechanism of action is still not completely understood, U.S. research in these areas supported the lack of significant activity of modafinil in the nucleus accumbens (which is activated by CNS stimulants associated with a potential for abuse) and selectivity for hypothalamic areas associated with the facilitation of "wake promotion."

II. The U.S. Clinical Trials of Modafinil in Narcolepsy

The U.S. Modafinil in Narcolepsy Multicenter Study Group conducted phase 3 clinical trials for modafinil in narcolepsy (7,8). The two double-blind, placebo-controlled U.S. studies in narcolepsy were similarly designed, with identical end points that measured both objective and subjective sleepiness, as well as overall clinical condition (7,8). Each trial (one conducted at 18 centers and one at 21 centers) used an objective measure of physiologic excessive sleepiness, the Maintenance of Wakefulness Test (MWT) (9). as a primary end point. The Clinical Global Impression of Change (CGI-C) was also used as a primary end point to assess subjective improvements in clinical condition and response or lack of response to modafinil. The Multiple Sleep

Latency Test (MSLT) was used as a secondary end point (10), as was the Epworth Sleepiness Scale (ESS) (11), an eight-item, patient-completed Likert scale that has become widely adopted as a subjective assessment tool in sleep research.

Each narcolepsy trial was 9 weeks in duration, with clinic visits scheduled every 3 weeks (nocturnal polysomnography was performed the night before each clinic visit to assess sleep efficiency and changes in sleep architecture). The studies were sufficiently powered to detect improvements in the two primary end points, changes from baseline in mean MWT sleep latency, and CGI disease severity with modafinil versus placebo. The efficacy analyses used an intent-to-treat population (patients who received at least one dose of study drug and had at least one postbaseline assessment on both the MWT and CGI-C) (7,8).

A total of 558 patients were included in the efficacy analyses of these trials, and randomized to placebo, modafinil 200 mg, or modafinil 400 mg. The baseline demographics and disease severity were similar between these studies, and consistent with those of narcoleptics in the general U.S. population. There was a delay of approximately 15 years between the first onset of symptoms and a clinical diagnosis, a common finding among U.S. narcolepsy patients. The majority of participants (81% in both studies combined) also had cataplexy. Patients who could not discontinue anticataplectic medications safely for the trial periods were excluded from participation). The maximum mean MSLT sleep latency was 3.3 minutes in any of the six treatment groups, below the 5 minutes adopted by the *International Classification of Sleep Disorders: Revised Diagnostic and Coding Manual* (*ICSD*) as a cutoff value for severe excessive sleepiness (12). The mean ESS scores ranged from 17.1 to 18.3, at least equal to those seen in narcolepsy populations who were evaluated during the initial validation of the ESS (11). All patients except one were at least mildly ill on the Clinical Global Impression of Severity (CGI-S), with about one third considered markedly to extremely ill.

The first study conducted, the 18-center study, used only a 1-day titration period for the 400-mg group (both groups received 200 mg on day 1). A higher percentage of patients in the 400-mg group withdrew due to adverse events compared with the 200-mg and placebo groups (12% vs 1% and 0%, respectively) (7). Therefore, the subsequent 21-center study used a more refined step-up protocol, giving each active treatment group 100 mg of modafinil on days 1 through 7 and 200 mg on day 8. Starting on day 9, patients in the 400-mg group were moved to the higher dose. Only 1% of patients in the 400-mg group withdrew due to adverse events; the lower incidence was likely (but not definitively) attributable to the longer titration schedule (8).

The 21-center study also included a 2-week discontinuation period to determine whether abrupt discontinuation of modafinil was associated with adverse events (especially those related to withdrawal from CNS stimulants, including fatigue, insomnia, increased appetite, and agitation). In this phase, those receiving placebo remained on placebo, while 80% of those in the modafinil groups switched over to placebo at the end of week 9. The remaining 20% continued on modafinil to serve as a comparator group (8).

The combined results of these studies on the primary end points are displayed in Figure 1, with the secondary as well as primary end points included in Table 1 (7,8). Mean sleep latency on the MWT increased by more than 2 minutes in each treatment group, compared with a decrease of 0.7 minutes in the placebo group ($P < .001$, change

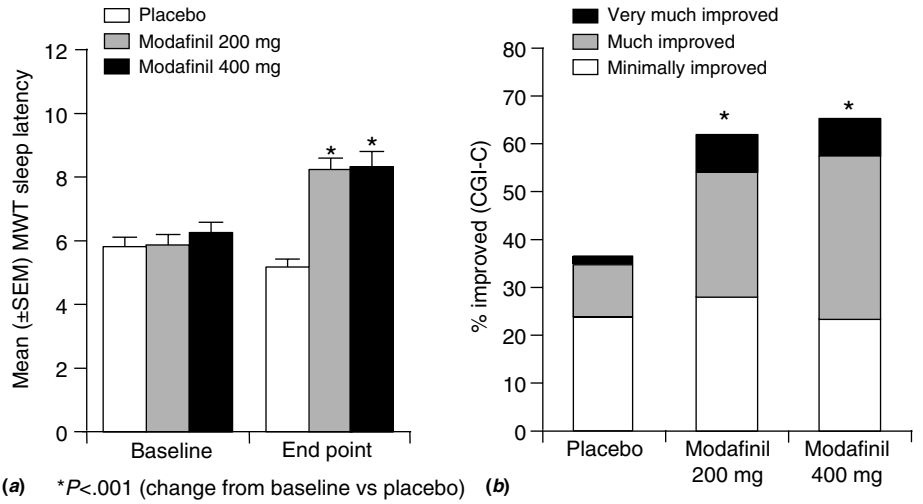


Figure 1 Combined data from the 18- and 21-center narcolepsy studies, showing results from the two primary outcome measures: (***a***) change in sleep latency on the Maintenance of Wakefulness Test (MWT); and (***b***) improvement in overall clinical condition on the Clinical Global Impression of Change (CGI-C). *Source*: From Refs. 7,8.

from baseline vs placebo for both doses). A significantly higher percentage of modafinil patients showed improvement in overall clinical condition on the CGI-C (61-66%, versus 37% for placebo; $P < .001$). Similar objective improvements were seen on the MSLT, and subjective improvements on the ESS (7,8). During the discontinuation period of the 21-center study, no symptoms consistent with tolerance or abrupt withdrawal were observed. However, in those who discontinued modafinil, improvements in wakefulness seen over the course of the study were lost on both subjective (ESS) and objective (MWT) measures (8). Polysomnographic recordings showed no significant changes in sleep efficiency (i.e., the time asleep as a percent of the total time in bed), time spent in REM sleep, or time spent in each non-REM sleep stage (7,8).

Modafinil was considered generally well tolerated in these studies; the most common adverse event was headache (36-54% of patients), the majority of which occurred in the first month of treatment and resolved within a few days. There was no difference in treatment response among those with or without cataplexy. Because those who could not discontinue anticataplectic medications were excluded from the studies, however, it is likely that patients with severe cataplexy were underrepresented (a potential limitation of these studies). The incidence of cataplexy as an adverse event ranged from 1 to 4%, with no difference between the treatment and placebo groups. There is no evidence that modafinil has any effect on helping cataplexy or the other ancillary features of narcolepsy, such as hypnagogic hallucinations or sleep paralysis.

The findings of the U.S. Modafinil in Narcolepsy Multicenter Study Group are notable for several reasons, including the consistency of response on the same measures between trials, and the consistent improvement across different measures within each trial. The absolute changes from baseline on the MWT and MSLT may appear

Table 1 Mean Change From Baseline (+SD) in Primary and Secondary Outcome Measures in the Placebo-Controlled Studies of Modafinil in Narcolepsy, OSAHS, and SWSD

	Narcolepsy (9 week, combined results)			OSAHS (12 week)			OSAHS (4 week)		SWSD (12 week)		SWSD (12 week safety)		
Dose (mg)	200	400	Pbo	200	400	Pbo	400	Pbo	200	Pbo	200	300	Pbo
MWT sleep latency, min	2.3 (4.6)[a]	2.1 (4.8)[a]	−0.7 (4.39)	1.6 (4.8)[a]	1.5 (5.0)[a]	−1.1 (4.6)	NA	NA	NA	NA	NA	NA	NA
MSLT sleep latency, min	1.9 (3.4)[b]	2.1 (3.7)[a]	0.9 (2.8)	NA	NA	NA	1.0 (3.7)[c]	−0.2 (4.1)	1.7 (3.8)[b]	0.3 (2.8)	NA	NA	NA
CGI-C, percent improved[d]	61[a]	66[a]	37	60[a]	68[a]	37	66[c]	34	74[a]	36	NA	NA	NA
ESS score	−3.9 (4.8)[a]	−5.0 (5.0)[a]	−1.5 (3.6)	−4.5 (4.7)[a]	−4.7 (4.3)[a]	−1.8 (3.5)	−4.6 (4.3)[a]	-2.0 (3.6)	NA	NA	NA	NA	NA
KSS score	NA	NA	NA	NA	NA	NA	NA	NA	−1.5 (1.5)[a]	−0.4 (1.5)	NA	NA	NA
PVT lapses of attention	NA	NA	NA	−2.8 (6.5)[a]	−0.8 (3.5)[c]	−2 (5.0)	NA	NA	−3.8 (21.0)[b]	7.2 (20.8)	NA	NA	NA
FOSQ total score	NA	NA	NA	1.9 (2.5)[b]	2.1 (2.6)[b]	0.8 (1.9)	1.8 (2.2)[b]	1.2 (2.0)	1.3 (2.3)	1.0 (2.6)	2.0 (2.8)	2.3 (2.7)[c]	1.6 (2.7)
SF-36 mental composite score	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.7 (8.1)[c]	3.2 (7.6)[c]	0.7 (11.2)

[a]$P < 0.001$ vs. placebo; [b]$P < 0.01$ vs placebo; [c]$P < 0.05$ vs. placebo.
[d]Results represented the percent of patients improved from baseline.
Abbreviations: NA, not applicable (measurement scale not used in particular study); MWT, Maintenance of Wakefulness Test; MSLT, Multiple Sleep Latency Test; CGI-C, Clinical Global Impression of Change; ESS, Epworth Sleepiness Scale; KSS, Karolinska Sleepiness Scale; PVT, Psychomotor Vigilance Task; FOSQ, Functional Outcomes of Sleep Questionnaire; SF-36, Medical Outcomes Survey-Short Form 36.
Source: From Refs. 1,7,8,29.

small; however, these tests are conducted in a controlled laboratory setting designed to maximize the likelihood of sleep onset (i.e., in a darkened room while laying semirecumbent) (9,10). Under these conditions, small increases in sleep latency (e.g., 1–2minutes) can represent clinically significant improvements in wakefulness (13). (It should be noted, however, that modafinil did not completely resolve ES in these profoundly sleepy populations; mean sleep latency, although significantly improved, was still considered to be in the mild to moderate range.) (7,8)

The results of the U.S. studies corroborate those by a group of Canadian investigators in a randomized, double-blind, placebo-controlled, 6-week trial. In this trial, consisting of three 2-week crossover phases, significant improvements were seen on the MWT and ESS at 200- and 400-mg doses, given twice daily in the morning and at noon (14). (None of these three trials showed a dose-response relationship.)

The discontinuation results in the 21-center U.S. trial were also supported by similar results from a 16-week open-label extension of the Canadian study, which ended with a 2-week, double-blind, abrupt discontinuation period (15). Together, the U.S. and Canadian studies formed the basis for recommending modafinil as a standard of care in a 2000 update to the American Academy of Sleep Medicine guidelines for narcolepsy treatment (16). The standard-of-care designation (only assigned to modafinil) reflected the favorable risk/benefit profile of modafinil in these three studies, as well as several supporting studies conducted in the U.S. and France (17,18). Modafinil has now become the "first line" treatment for excessive sleepiness in most patients newly diagnosed with narcolepsy.

III. Expanding the Indications for Modafinil

The approval of modafinil marked the introduction in the U.S. of an agent with wake-promoting efficacy similar to that of CNS stimulants, but with a more favorable safety profile and a lower potential for abuse and/or dependence. This lower abuse potential is reflected by its schedule IV labeling in the Controlled Substances Act (19). Under the Act, the U.S. Attorney General's office classifies drugs according to five schedules based on their potential for abuse, any history or pattern of prior abuse, their chemical properties (e.g., their ability to be injected or smoked as opposed to taken orally), and/or their pharmacokinetic activity (e.g., whether a drug metabolizes into a substance already listed under the Act). The two other agents approved in the U.S. for treating narcolepsy-related sleepiness, amphetamine and methylphenidate, are schedule II substances, denoting a high potential for abuse. (A third CNS stimulant, pemoline, is listed with modafinil in schedule IV, although it is not specifically approved for narcolepsy.) In addition, the pemoline prescribing information was updated in 1999 to include a "black box" warning on the potential for severe hepatotoxicity.)

Approximately 1.4 million prescriptions for modafinil were written in the U.S. in 2003. More than two thirds of all prescriptions for excessive sleepiness in narcolepsy were for modafinil; however, a significant percentage of overall modafinil prescriptions were for indications other than narcolepsy. According to prescription audits, most prescription activity for modafinil in the U.S. was generated by psychiatrists (36%), followed by neurologists (22%), primary care physicians (16%), and sleep medicine specialists/pulmonologists (13%) (20,21,22). This pattern of use is likely driven by a

number of factors, including the desire to assess the efficacy of modafinil for excessive sleepiness in diseases other than narcolepsy; a need for effective medications to treat related symptoms, such as fatigue; and a search for additional effects on signs and symptoms that may or may not be directly related to sleepiness, such as cognitive processing, executive functioning, memory, and attention.

A number of U.S.-based investigations have assessed the efficacy of modafinil for treating excessive sleepiness associated with Parkinson's disease, depression, schizophrenia, traumatic brain injury, and stroke, and fatigue in multiple sclerosis and depression (23,24,25,26,27,28). The majority of these studies have reported improvements in sleepiness and/or fatigue, although most have been designed as smaller pilot studies or single-blind trials; few have been randomized trials with a double-blind, placebo-controlled design. In addition, some studies on the treatment of excessive sleepiness and fatigue in patients with depression have shown significant improvement with the use of selective serotonin reuptake inhibitors (SSRIs) plus modafinil in the earlier measurement periods (e.g., weeks 1-2), but no significant difference at later measurement periods. This has generally not been due to a deterioration of efficacy with modafinil, but subsequent improvements in efficacy in groups receiving SSRIs plus placebo.

In January 2004, the U.S. indication for modafinil was expanded to include excessive sleepiness associated with obstructive sleep apnea/hypopnea syndrome (OSAHS) and shift work sleep disorder (SWSD). (In the OSAHS populations, modafinil is indicated as an adjunct to standard treatments for the underlying obstruction in those who continue to experience excessive sleepiness despite treatment of the obstruction.) Two randomized, placebo-controlled studies were conducted for each of these disorders. The results of these six trials are presented in Table 1 (1,7,8,29). Like the narcolepsy studies, the two OSAHS studies and the 12-week efficacy study of SWSD included a number of objective and subjective assessments of excessive sleepiness, including the Karolinska Sleepiness Scale (KSS), which was used in the 12-week efficacy study of SWSD because of its proven utility for assessing excessive sleepiness in circadian rhythm sleep disorders (30). (The second SWSD trial was designed primarily to assess safety and quality of life, and did not include end points directly related to improvements in wakefulness) The OSAHS and SWSD studies also used the Psychomotor Vigilance Task (PVT) to measure the participants' ability to sustain attention, given the association between excessive sleepiness and lapses of attention (31). The improvements in wakefulness and overall clinical condition in the OSAHS and SWSD trials were consistent with those in the narcolepsy trials, with significant improvements found at both doses between baseline and end point (1,7,8,29).

IV. U.S. Safety and Long-Term Efficacy Data with Modafinil

Substantial safety data on the use of modafinil in the U.S. have been compiled through a number of sources, including adverse event monitoring in the clinical trials, postmarketing adverse event reporting, preclinical studies on drug interactions, studies on abuse and dependence potential, and active postmarketing surveillance programs. Overall, modafinil exhibited a favorable adverse event profile in the U.S. placebo-controlled studies (1,7,8,29). The most common adverse event was headache; most were mild or moderate

in severity, appeared in the early stages of therapy, and resolved within several days. A dose-response relationship was seen with headache and anxiety. Anecdotally, in clinical practice, headaches and nervousness that can occur with modafinil can be helped by reducing the dosage of modafinil for a few days before resuming the prior dosage. If the symptoms are mild, then maintaining the starting dose for a few days before increasing the dose again, can be helpful. Expanded analyses that included all studies in narcolepsy, OSAHS, and SWSD, as well as other disorders (e.g., depression and attention deficit hyperactivity disorder), showed adverse event patterns similar to those in the six primary placebo-controlled studies (Table 2) (32).

Neither long-term, open-label studies nor postmarketing adverse event reporting have revealed patterns of adverse events that differ from those in the double-blind, placebo-controlled studies. Patients in the narcolepsy studies were enrolled in an open-label extension study using flexible doses of 200 to 400 mg; results through 136 weeks have demonstrated continued improvement in wakefulness on the ESS, and expected treatment-emergent adverse events including headache, nervousness, and nausea (1,33). Similar results have been seen in open-label extensions of the OSAHS and SWSD trials (1,34). The incidence of cataplexy deemed related to modafinil treatment in the first 40 weeks of open-label treatment was 2.7%. A total of 28.7% of patients discontinued treatment, for reasons that included "insufficient efficacy" (11.5%) and adverse events (9%). Postmarketing adverse-event reporting has revealed isolated cases of agranulocytosis, symptoms of psychosis, urticaria, and angioedema. Due to the difficulties inherent in spontaneous reporting of adverse events, however, reliable estimates of the prevalence of these events cannot be made.

Table 2 Adverse Events Across Primary Disorders of Sleep and Wakefulness in Studies of Modafinil[a]

	Placebo-controlled Studies		All narcolepsy, OSAHS, SWSD	All studies
Adverse event (%)	Modafinil ($n = 934$)	Placebo ($n = 567$)	Modafinil ($n = 2094$)	Modafinil ($n = 3744$)
Headache[b]	34	23	29	24
Nausea	11	3	10	9
Nervousness	7	3	9	10
Rhinitis	7	6	5	6
Back pain	6	5	6	4
Diarrhea	6	5	6	4
Anxiety[b]	5	<1	6	7
Dizziness	5	4	5	4
Dyspepsia	5	4	6	4
Insomnia	5	1	7	9

[a]5% is for modafinil treatment (doses combined) and placebo. Diseases studied include narcolepsy, idiopathic hypersomnia, OSAHS, SWSD, major depressive disorder, and attention deficit-hyperactivity disorder.
[b]Dose-response relationship observed.
Abbreviations: OSAHS, obstructive sleep apnea/hypopnea syndrome; SWSD, shift work sleep disorder.
Source: From Refs. 1,7,8,29,32.

U.S.-based pharmacokinetic analyses tested modafinil in conjunction with a number of drugs that are also metabolized by the hepatic cytochrome P450 enzyme system, including oral contraceptives, warfarin, and triazolam (35,36). There are no known adverse reactions with anticataplectic medications when taken concurrently. In patients who are CYP2D6 deficient an elevation of clomipramine levels can occur. Blood levels of fluoxetine may be increased slightly by modafinil. The anticataplectic medication, sodium oxybate, has been shown to provide additional benefit to daytime alertness when given with modafinil (Orphan Medical Inc. information on file.) Among the notable interactions was a decrease in the peak plasma concentrations of ethinyl estradiol. As a result, the prescribing information contains a precaution advising women to seek alternative or additional methods of contraceptive while taking modafinil and for 1 month following discontinuation.

Postmarketing surveillance of modafinil in the U.S. is being conducted by the Haight-Ashbury Free Clinics, a San Francisco-based network of clinics with experience in the rapid assessment of abuse patterns. The postmarketing surveillance program involves medical, media, addiction-treatment, and law enforcement sources. To date, these sources have not detected generalized interest in modafinil as a drug of abuse. However, there have been isolated cases of modafinil abuse reported through these methods of surveillance (37). In addition, U.S.-based clinical studies in persons experienced with drugs of abuse have demonstrated the modafinil can produce mild psychoactive and euphoric effects consistent with those of CNS stimulants (38,39). However, results are conflicting in this area, and the severity of these effects tend to be lower than those seen with CNS stimulants.

Continued interest will be on the use of modafinil in OSAHS patients in conjunction with nCPAP, and those at risk for hypertension. Long-term extension studies of CPAP use in OSAHS, but not short-term studies have shown statistically significant reductions in nCPAP use, ranging from approximately 20 minutes to a half hour (1,34). In addition, while mean systolic or diastolic blood pressure did not rise significantly in patients treated with modafinil, more patients taking modafinil in the placebo-controlled clinical studies required either an increase in dose or an additional prescription for an antihypertensive agent. The difference was more pronounced in the OSAHS patients, who are generally heavier and more prone to cardiovascular disease (32). Analyses of electrocardiographic features such as QTc intervals did not reveal any evidence of detrimental effects. The overall cardiovascular profile of modafinil is favorable compared with that of the stimulants.

V. Future Directions for Modafinil Research

Several U.S. studies have focused on determining optimal dosing protocols for modafinil in narcolepsy patients, including the use of doses higher than the recommended dose of 200 mg, as well as split dosing to achieve improvements in evening wakefulness. While no dose-response effect was seen for the 400-mg dose compared with 200 mg in the placebo-controlled clinical studies, the first MWT was generally performed an hour after dosing of modafinil, too early for the agent to reach peak plasma concentrations. A more recent study employed a modified version of the MWT that included evening test sessions. This study demonstrated an improved

response to the 400-mg dose compared with 200 mg, whether it was given as a single morning dose or a split dose in the morning and at noon. The greatest improvements in evening wakefulness were seen with the split-dose regimen (40). More recently, a 600-mg split-dose regimen (400 mg in the morning and 200 mg in the early afternoon) was found to achieve more consistent wakefulness throughout the day (morning, afternoon, and evening) compared with 200 or 400 mg qAM or a 400 mg split-dose regimen (41). Anecdotally, in clinical practice some physicians have reported increased favorable responses with doses up to 1200 mg/day. This contrasts with research studies on fatigue, where significant improvements have not been seen consistently with doses higher then 200 mg/day (26).

Dosing studies have also addressed considerations involved in switching from CNS stimulants to modafinil. A recent study of 151 patients showed that modafinil can improve daytime wakefulness in patients who have used previous CNS stimulant therapy (42). Additional data have shown that patients may be safely switched from methylphenidate both with, and without, a short washout or dose titration period (43). European data, however, have shown greater difficulty in switching from amphetamine, with some cases of failure to withdraw reported (44).

Concern has been expressed by a number of groups, including physicians and addiction-treatment professionals, that modafinil may be exploited as a "lifestyle" drug by healthy persons who simply want more time to work or engage in all-night social activities. The immediate postmarketing surveillance has not supported this concern, but increasing media exposure in the U.S., including reports of improved performance in those subjected to extended periods of sleep deprivation (e.g., military personnel) may fuel interest in the wake-promoting properties of modafinil and subsequent "lifestyle" use.

VI. Summary

Modafinil was approved by the United States Food and Drug Administration (U.S. FDA) in December 1998 for excessive sleepiness associated with narcolepsy, and remains the only nonsympathomimetic agent in the U.S. approved for this indication. Two randomized, placebo-controlled studies in the U.S. involving more than 550 patients demonstrated significant efficacy for modafinil on both objective and subjective measures of sleepiness, with mean increases in sleep latency of more than 2 minutes on the Maintenance of Wakefulness Test (MWT) and mean decreases in Epworth Sleepiness Scale (ESS) scores of approximately 5 points. In January 2004, the indication for modafinil was expanded to include the treatment of excessive sleepiness associated with obstructive sleep apnea/hypopnea syndrome (OSAHS) and shift work sleep disorder (SWSD), based on three additional U.S. studies showing improvements in wakefulness comparable to those seen in the narcolepsy populations. Modafinil has become the most widely prescribed agent in the U.S. for excessive sleepiness associated with narcolepsy, comprising approximately 70% of the U.S. market for this symptom. Other medications may be required to treat the ancillary symptoms of narcolepsy such as cataplexy. Current U.S. research is exploring optimal dosing regimens and the use of modafinil for improving signs and symptoms that are distinct from but may be related to excessive sleepiness, including fatigue, depressed mood, and impairments in attention.

References

1. Cephalon, Inc.; data on file, 2004.
2. Bastuji H, Jouvet M. Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Prog Neuropsychopharmacol Biol Psychiatry 1988; 12:695–700.
3. Federal Food, Drug, and Cosmetic Act, 21 USC §§ 360aa-360ee.
4. Robertson P Jr, Hellriegel ET. Clinical pharmacokinetic profile of modafinil. Clin Pharmacokinet 2003; 42:123–137.
5. Scammell TE, Estabrooke IV, McCarthy MT, et al. Hypothalamic arousal regions are activated during modafinil-induced wakefulness. J Neurosci 2000; 20:8620–8628.
6. Robertson P, Decory HH, Madan A, Parkinson A. In vitro inhibition and induction of human hepatic cytochrome P450 enzymes by modafinil. Drug Metab Disposition 2000; 28:664–671.
7. U.S. Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol 1998; 43:88–97.
8. U.S. Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology 2000; 54:1166–1175.
9. Hartse KM, Roth T, Zorick FJ. Daytime sleepiness and daytime wakefulness: the effect of instruction. Sleep 1982; 5:S107–S118.
10. Thorpy MJ. The clinical use of the Multiple Sleep Latency Test. Sleep 1992; 15:268–276.
11. Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep 1991; 14:540–545.
12. The International Classification of Sleep Disorders, Revised. Diagnostic and Coding Manual. Rochester, Minnesota: American Sleep Disorders Association, 1997.
13. Patel SR, White DP, Malhotra A, Stanchina ML, Ayas NT. Continuous positive airway pressure therapy for treating sleepiness in a diverse population with obstructive sleep apnea: results of a meta-analysis. Arch Intern Med 2003; 163:565–571.
14. Broughton RJ, Fleming JAE, George CFP, Hill JD, Kryger MH, Moldofsky H, Montplaiser JY, Morehouse RL, Moscovitch A, Murphy WF. Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. Neurology 1997; 49:444–451.
15. Moldofsky H, Broughton RJ, Hill JD. A randomized trial of the long-term, continued efficacy and safety of modafinil in narcolepsy. Sleep Med 2000; 1:109–116.
16. Littner M, Johnson SF, McCall WV, Anderson WM, Davila D, Hartse K, Kushida CA, Wise MS, Hirshkowitz M, Woodson BT, for the American Academy of Sleep Medicine Standards of Practice Committee. Practice parameters for the treatment of narcolepsy: an update for 2000. Sleep 2001; 24:451-466.
17. Besset A, Chetrit M, Carlander B, Billiard M. Use of modafinil in the treatment of narcolepsy: a long term follow-up study. Neurophysiol Clin 1996; 26:60–66.
18. Billiard M, Besset A, Montplaisir J, Laffont F, Goldenberg F, Weill JS, Lubin S. Modafinil: a double-blind multicentric study. Sleep 1994; 17(8 Suppl):S107–S112.
19. Controlled Substances Act. 21 USCS §811; 21 CFR §1308.
20. IMS Health, Inc.; National Prescription Audit Plus; June 2004.
21. NDC Prescriber data. NDC Health; June 2004.
22. Verispan Physician Drug and Diagnosis Audit (PDDA); March 2004.
23. DeBattista C, Doghramji K, Menza MA, Rosenthal MH, Fieve RR. Adjunct modafinil for the short-term treatment of fatigue and sleepiness in patients with major depressive disorder: a preliminary double-blind, placebo-controlled study. J Clin Psychiatry 2003; 64:1057–1064.
24. Elovic E. Use of Provigil for underarousal following TBI. J Head Trauma Rehabil 2000; 15:1068–1071.
25. Adler CH, Caviness JN, Hentz JG, Lind M, Tiede J. Randomized trial of modafinil for treating subjective daytime sleepiness in patients with Parkinson's disease. Mov Disord 2003; 18:287.
26. Rammohan KW, Rosenberg JH, Lynn DJ, Blumenfeld AM, Pollak CP, Nagaraja HN. Efficacy and safety of modafinil (Provigil) for the treatment of fatigue in multiple sclerosis: a two centre phase 2 study. J Neurol Neurosurg Psychiatry 2002; 72:179.
27. Rosenthal MH, Bryant SL. Benefits of adjunct modafinil in an open-label, pilot study in patients with schizophrenia. Clin Neuropharmacol 2004; 27:38–43.
28. Smith BW. Modafinil for treatment of cognitive side effects of antiepileptic drugs in a patient with seizures and stroke. Epilepsy Behav 2003; 4:352–353.

29. Pack AI, Black JE, Schwartz JRL, Matheson JK, for the U.S. Modafinil in Obstructive Sleep Apnea Study Group. Modafinil as adjunct therapy for daytime sleepiness in obstructive sleep apnea. Am J Respir Crit Care Med 2001; 164:1675–1681.
30. Akerstedt T. Subjective and objective sleepiness in the individual. Intern J Neurosci 1990; 52:29–37.
31. Jewett ME, Dijk DJ, Kronauer RE, Dinges DF. Dose-response relationship between sleep duration and human psychomotor vigilance and subjective alertness. Sleep 1999; 22:171–179.
32. Thorpy MJ, Black J, Erman MK. The safety profile of modafinil in disorders of sleep and wakefulness. Neurology 2004; 62 (suppl 5):A56–A57.
33. Mitler MM, Harsh J, Hirshkowitz M, Guilleminault C, for the U.S. Modafinil in Narcolepsy Multicenter Study Group. Long-term efficacy and safety of modafinil (PROVIGIL®) for the treatment of excessive daytime sleepiness associated with narcolepsy. Sleep Med 2000; 1:231–243.
34. Schwartz JRL, Hirshkowitz M, Erman MK, Schmidt-Nowara W, for the U.S. Modafinil in Obstructive Sleep Apnea Study Group. Modafinil as adjunct therapy for daytime sleepiness in obstructive sleep apnea: a 12-week, open-label study. CHEST 2003; 24:2192–2199.
35. Robertson P Jr, Hellriegel ET, Arora S, Nelson M. Effect of modafinil on the pharmacokinetics of ethinyl estradiol and triazolam in healthy volunteers. Clin Pharmacol Ther 2002; 71:46–56.
36. Robertson P Jr, Hellriegel ET, Arora S, Nelson M. Effect of modafinil at steady state on the single-dose pharmacokinetic profile of warfarin in healthy volunteers. J Clin Pharmacol 2002; 42:205–214.
37. Smith DE, Calhoun SR, Galloway GP, Romanoff SJ, Wolfe NE. Postmarketing surveillance of modafinil abuse and misuse (abstract). Sleep 2004; 27(suppl):A57.
38. Rush CR, Kelly TH, Hays LR, Baker RW, Wooten AF. Acute behavioral and physiological effects of modafinil in drug abusers. Behav Pharmacol 2002; 13:105–115.
39. Jasinski DR, Kovacevic-Ristanovic R. Evaluation of the abuse liability of modafinil and other drugs for excessive daytime sleepiness associated with narcolepsy. Clin Neuropharmacol 2000; 23:149–156.
40. Schwartz JRL, Feldman NT, Bogan RK, Nelson MT, Hughes RJ. Dosing regimen effects of narcolepsy for improving daytime wakefulness in patients with narcolepsy. Clin Neuropharmacol 2003; 26:252–257.
41. Schwartz JRL, Feldman NT, Bogan RK. Dose response and dose regimen effects of modafinil in sustaining daytime wakefulness in narcolepsy patients with residual excessive sleepiness. J Neurol Clin Neurosci 2005. In press.
42. Schwartz JRL, Feldman NT, Fry JM, Harsh J. Efficacy and safety of modafinil for improving daytime wakefulness in patients treated previously with psychostimulants. Sleep Med 2003; 4:43–49.
43. Thorpy MJ, Schwartz JR, Kovacevic-Ristanovic R, Hayduk R. Initiating treatment with modafinil for control of excessive daytime sleepiness in patients switching from methylphenidate: an open-label safety study assessing three strategies. Psychopharmacology (Berl) 2003; 167:380–385.
44. Guilleminault C, Aftab FA, Karadeniz D, Philip P, Leger D. Problems associated with switch to modafinil-a novel alerting agent in narcolepsy. Eur J Neurol 2000; 7:381–384.

53
Molecular and Cellular Actions of γ-Hydroxybutyric Acid: Possible Mechanisms Underlying GHB Efficacy in Narcolepsy

XINMIN S. XIE
Biosciences Division, SRI International, Menlo Park, California, U.S.A.

DANIEL PARDI
Jazz Pharmaceuticals, Inc., Palo Alto, California, U.S.A.

JED BLACK
Stanford University, Sleep Disorders Clinic, Stanford, California, U.S.A.

I. Introduction

γ-Hydroxybutyric acid (GHB) is a naturally occurring endogenous substance that functions as an inhibitory neurotransmitter / neuromodulator in the central nervous system in mammals. Endogenous GHB is synthesized and released in the brain by specific neuronal pathways implicated in the control of the GABAergic, dopaminergic, and noradrenergic systems. Interestingly, GHB was initially synthesized some 40 years ago to obtain a GABAergic substance that penetrates the brain freely. Since then, GHB has been used in humans for its therapeutic applications in anesthesia, sleep disorders—particularly narcolepsy, fibromyalgia, and movement disorders. However, this compound also has a complicated history of abuse issues. The mechanism of action of GHB is still poorly understood. Current opinion is that GHB at low micromolar concentrations exerts its modulations likely through the GHB high-affinity binding sites, that is, GHB-specific receptors. One of these receptors has been recently cloned from rat brain hippocampus and is thought to regulate GABAergic activities via a subtle balance between sensitized and desensitized states. In contrast, GHB at millimolar concentrations directly activates $GABA_B$ receptors, reducing neurotransmitter release presynaptically, and causing membrane hyperpolorazation and a decrease in input resistance postsynaptically. Exogenously administered GHB does not induce sedation, hypothermia and muscle relaxation in $GABA_{B(1)}$ receptor knockout (KO) mice, although GHB binding sites are unaltered. This was the first study to provide solid molecular evidence of the critical involvement of $GABA_B$ receptors in the GHB pharmacological actions. The $GABA_B$-mediated inhibition in the brain, particularly directly acting on the excitatory hypocretin/orexin neurons in the lateral hypothalamus, may underlie GHB selective promotion of non-rapid eye movement sleep (NREM) and sleep consolidation observed in humans and animals. However, mechanisms for GHB efficacy in the treatment of excessive sleepiness and cataplexy in narcoleptic patients remain elusive. In

this review, we have focused on molecular and cellular actions of GHB in an attempt to provide insight into possible mechanisms relevant to the treatment of narcolepsy.

γ-Hydroxybutyric acid (GHB, Fig. 1) is a four-carbon fatty acid, which is principally metabolized from the inhibitory neurotransmitter γ-aminobutyric acid (GABA) by the substitution of a hydroxyl group for the amine via a reductive

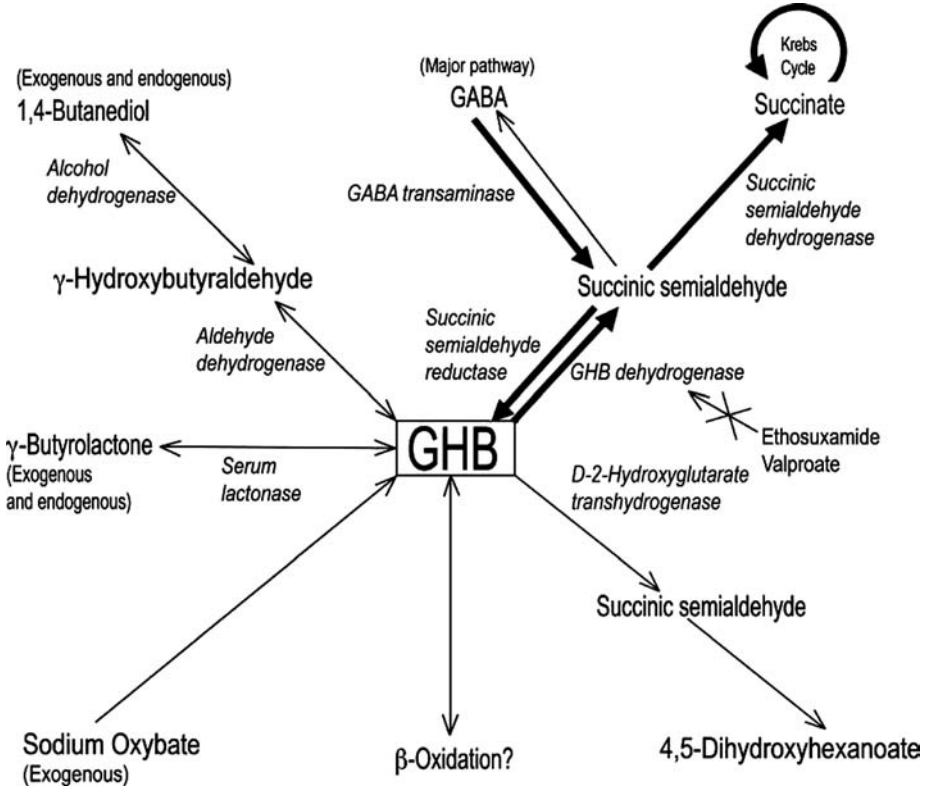


Figure 1 Bold black lines represent major endogenous pathways. *Synthesis*: The major synthesis pathway for GHB begins with GABA converting to succinic semialdehyde (SSA) via mitochondial GABA transaminase. SSA is metabolized to GHB via cytosolic SSA reductase. Other endogenous precursors include: 1,4-Butanediol (I,4-BD), gamma-butyrolactone (GBL), and possibly beta-oxidation. 1,4-BD converts to gamma-hydroxybutyraldehyde via the alcohol dehydrogenase enzyme; gamma-hydroxybutyraldehyde is then converted via aldehyde dehydrogenase to GHB. GBL is converted to GHB via circulating lactonases; which are not present in brain tissue. ß-Oxidation is putative. Exogenous precursors include, 1,4-BD, GBL, and sodium oxybate. Sodium oxybate is the sodium salt of GHB. When ingested, the sodium ion dissociates producing GHB. Bold lines represent major catabolic pathways for GHB.
Catabolism: The major catabolic pathway for GHB is oxidation to succinic semialdehyde via GHB dehydrogenase. SSA is metabolized mostly to succinate via SSA dehydrogenase; the enzyme missing in the pathology of GHB aciduria. A mitochondrial GHB transhydrogenase has been shown to catabolize GHB to SSA with 4,5-dihydroxyhexanoate as the end product. Again, ß-oxidation is putative and controversial.

pathway in the brain. Natural GHB at micromolar concentration was found in both brain (1,2) and peripheral tissues (3). Synthetic sodium oxybate is the sodium salt of γ-hydroxybutyrate [$HO(CH_2)_3COONa$, Fig. 1] and is used for exogenous oral administration of GHB.

Initial evaluation of GHB in animals and humans explored its sleep-promoting, and anesthesia-inducing capacity. This substance was recognized to preferentially promote non-rapid eye movement sleep (NREM, or slow-wave deep sleep), and facilitate sleep consolidation. These properties led to the exploration of its utility in a variety of clinical indications. In particular, the majority of recent therapeutic research has focused on narcolepsy.

Since in this chapter we focus on the mechanism of actions of GHB, for simplicity, the name GHB will be mostly used throughout the chapter including when referring to literature wherein authors have used another name.

Other potential therapeutic effects of GHB have been explored. These include reduction of intracranial pressure (4,5); tissue-sparing and neuroprotective effects in ischemia-induced challenges (6–11), relief of pain, anxiety and tension during childbirth (12); impact on anxiety conditions (13); treating alcohol withdrawal syndrome (14–22); treating heroin dependence (23,24); efficacy in improving the pain, fatigue and sleep fragmentation of fibromyalgia syndrome (25,26); utility as a antidepressant (27); reducing negative effect of cocaine self-administration (28); and hypnotic potential in healthy subjects (29) and in those with insomnia (30) and Parkinson's disease and other hyperkinetic disorders (31–33). In addition, GHB also became known as a recreational drug of abuse (34–36).

In animal studies, pharmacologically induced supraphysiologic concentrations of GHB increases slow wave sleep, but also produces hypothermia and a variety of abnormal behaviors including hypolocomotion, catalepsy, ataxia, and loss of righting (37–40). Furthermore, GHB causes a constellation of EEG and behavioral changes in animals, which has been explored as an experimental model of generalized absence seizures (41–43). How GHB modulates the central nervous system, and thus behavior, is currently not fully understood. In the past decade, the mechanisms underlying the broad effects of GHB, and specifically the mechanisms responsible for the symptom amelioration of narcolepsy, has become of great interest for basic and clinical research. This chapter reviews basic neurochemistry and neurobiology of GHB, particularly its molecular and cellular actions, in an attempt to gain the insight into mode of actions of GHB underlying its efficacy in the treatment of narcolepsy. Many recent reviews on molecular and cellular actions of GHB have recently appeared (35,44,45).

II. Basic GHB Neurochemistry

A. GHB Formation

GHB was first synthesized in an attempt to create a GABA analogue that would mimic the function of GABA, but be less rapidly metabolized and, desirably, readily cross the blood brain barrier (46,47). A few years later it was found that GHB is an naturally occurring compound both in the brain (1,48) and peripheral tissues (3). The metabolic pathways of synthesis and degradation of GHB have been extensively studied in rat brain. It has been demonstrated that GHB is mainly formed from GABA in vivo (49).

GABA is transaminated to succinic semialdehyde (SSA), which is further reduced to GHB by reductases (Fig. 1). Different NADPH-and NADH-dependent SSA reductases responsible for GHB formation have been described (50).

Radio-label studies have shown that GABA is the major precursor of GHB in the brain (49,51). GHB formation occurs in GABAergic neurons or in neurons which synthesize GABA (52). About 0.05% (53) (in vitro) to 0.16% (in vivo) (49) of GABA is eventually metabolized to form GHB. In other words, the micromolar concentrations of GHB are approximately 0.1% of the millimolar concentrations GABA (54). GHB's synthesis from GABA occurs via the enzyme GABA-transaminase converting GABA to succinic semialdehyde (SSA) (55,56). SSA is reduced to form GHB through the enzyme SSA reductase (SSR), an aldehyde reductase enzyme with high affinity and specificity for SSA. This enzyme is mostly present in the cytosol and to a lesser extent in the synaptosomal fraction (53,57); a second catabolic pathway has also been described through a mitochondrial D-2 Hydroxyglutarate transaminase (58). However, evidence for this pathway is limited (59). Overall, glutamic acid is transformed to GABA by L-glutamic acid decarboxylase and the activity of this enzyme has been correlated to GHB formation in animal brain (60).

Another postulated precursor of endogenous GHB is 1,4-butanediol (1,4-BD) (61). Snead et al. (55) showed that in the rat brain, 1,4-BD is metabolized to g-hydroxybutyraldehyde by the enzyme alcohol dehydrogenase (ADH). Subsequently, gamma-hydroxybutryaldehyde is metabolized via aldehyde dehydrogenase to produce GHB. However, the formation of GHB from 1,4-BD in the brain appears to be insensitive to either pyrazole or ethanol, known inhibitors of the alcohol dehydrogenase (55,62). In addition to the synthesis of GHB in the brain, under certain conditions, the ADH present in the liver of animals and humans is also capable of the conversion of 1,4-BD to GHB. The peripheral GHB produced by the liver and other organs may enter the brain as it readily crosses the brain blood barrier (BBB).

Recently, the hypothesis that GHB undergoes carrier-mediated transport across the BBB has been proposed and tested using a rat in situ brain perfusion technique. Short-chain monocarboxylic acids (pyruvic, lactic, and beta-hydroxybutyric), medium-chain fatty acids (hexanoic and valproic), and organic anions (probenecid, benzoic, salicylic, and alpha-cyano-4-hydroxycinnamic acid) significantly inhibit GHB influx by 35 to 90%. In contrast, dicarboxylic acids (succinic and glutaric) and gamma-aminobutyric acid do not inhibit GHB BBB transport. These novel findings of GHB BBB transport suggest potential therapeutic approaches in the treatment of GHB overdoses (63).

Furthermore, naturally occurring endogenous γ-butyrolactone (GBL) in the brain is also another precursor of GHB. It was detected in rat brain at a concentration of 200 pmol/g tissue weight. Peripheral GBL is freely permeable across the blood-brain barrier (64,65), and it has been used as a pharmacological tool to produce a more consistent rat model of absence seizures compared to the use of GHB itself (66). However, the brain has very little capacity to either enzymatically convert the lactone to GHB or respond to the lactone itself (67).

B. GHB Concentrations

In mammals, GHB is heterogeneously present throughout the brain (1,2,68). Concentrations of GHB in the rodent brain are estimated at approximately 2–4 μM with

highest in the rat hippocampus and lowest in cerebellum (41,69,70). In brains of humans and monkeys, the highest GHB concentrations reach between 11–25 μM in the striatum (71). For comparative purposes, the highest reported GHB concentration in the periphery has been seen in brown fat at 37 μM (3). Localization of GHB within cytosolic and synaptosomal fractions (72) suggest a mechanism for presynaptic accumulation. The concentration of GHB is higher in the developing brain than in the adult brain (73). Moreover, GHB is found at millimolar concentrations in peripheral tissue such as heart, kidney, liver, muscle and brown fat (3). Peripheral GHB readily crosses the blood brain barrier; however, how the peripheral source influences the brain GHB concentration and function is unknown.

C. GHB Metabolism

Radio-isotope studies show that intraventricularly administered GHB is metabolized rapidly (74) into SSA (75,76). Studies using [3H]-GABA showed total brain turnover time of 26 min for GHB (49). GHB is catabolized by the SSA reductase enzyme (ALR1) in the presence of $NADP^+$ (77–79); this enzyme is now known as NADP-dependent GHB dehydrogenase (GHB-DH) (80). SSA and the co-substrate NADPH (81) are the products formed by GHB-DH from GHB and $NADP^+$ (77). This process is also coupled to the reduction of D-glucuronate which releases NADPH (82,83) accumulation and activates the pentose phosphate pathway (84). After GHB is converted into SSA, SSA is transformed to succinic acid, which is eventually metabolized to CO_2 and H_2O via the tricarboxcylic acid cycle (Krebs cycle). The enzyme GHB-DH is inhibited by various antiepileptic drugs (valproate, ethosuximide, barbiturates) and certain short-chain fatty acids (85). These compounds induce increased brain GHB levels by inhibiting GHB catabolism (53,85). The interaction between these antiepileptic drugs with the enzyme GHB-DH, resulting in an increase in the GHB and possibly GABA levels (see below) might contribute to their therapeutic effects, in addition to their commonly known mechanisms (e.g., modulations of ion channels and $GABA_A$ receptors).

Direct transport of GHB from cytoplasm into the mitochondria may also occur. A mitochondrial enzyme, D-2 Hydroxyglutarate transhydrogenase, is capable of reducing GHB to SSA suggesting that the mitochondria is one of locations of GHB catabolism (83,86).

In vivo experiments suggest that it is unlikely that GHB converts back to GABA (74,87,88); however, in vitro experimentation has shown that it is possible (87,89,90). It has been estimated that up to 0.36% of GHB could be metabolized into GABA in vitro (91). It has yet to be determined if this conversion is physiologically meaningful although it may also contribute to the unique modulation of pharmacological GHB. Another possible route for GHB degradation might be through beta-oxidation (92).

Under certain pathological conditions, brain GHB concentrations can dramatically increase. Most strikingly, succinate semialdehyde dehydrogenase (SSADH encoded by gene Aldh5a1) deficiency is a defect of GABA degradation that manifests in humans as GHB aciduria and a non-specific neurological disorder including psychomotor retardation, language delay, seizures, hypotonia and ataxia (93,94). To explore pathophysiology and develop new preclinical treatment approaches, Hogema et al., have developed a murine knockout model of SSADH ($Aldh5a1^{-/-}$) deficiency. Deficiency in SSADH resulted in brain GHB concentration increases to 120 – 240 μM.

Increased amounts of GHB and total GABA in urine were observed. The Aldh5a1$^{-/-}$ mice displayed ataxia and developed generalized seizures leading to immature death around postnatal 20 days (95,96).

III. Cellular Actions of GHB

A. The GHB Specific Binding Sites or GHB Receptors

The GHB specific binding site was first demonstrated using [3H]GHB in rat and human brain synaptosomal membranes. Two binding sites, a high affinity (30–580 nM (K_{d1}) and a low affinity (2.3–16 μM, K_{d2}) were shown to be saturable, pH dependent and linear with protein concentration (57,97,98). There is a distinct regional distribution of [3H]GHB binding sites in the brain, with the hippocampus being the richest and the cerebellum the poorest in density. Within the hippocampus, the highest density of GHB binding sites is found at the neuronal synapse (97). Some GHB binding sites have been shown to be located on cholinergic neurons and a subset population of GABAergic neurons that contain enkephalin immunoreactivity (99). The GHB distribution profile does not match those of $GABA_A$ or $GABA_B$ binding (37). Developmental studies have revealed the ontogeny of the GHB binding sites, indicating GHB binding is not demonstrable in rat brain until postnatal 18 days (100). In contrast, the GHB binding sites are absent from peripheral tissues like kidney, liver, muscle or heart of adult rats and tissues from adult humans (98), despite the fact that GHB is present in significant concentrations in these organs (53). GHB might function as a metabolic intermediate in the periphery (101). However, exogenously administered GHB greatly improves liver function and integrity after hypothermic preservation (102). While the mechanism underlying the effect of GHB is unknown, it is sure that no GHB binding sites are involved.

Furthermore, SSADH deficient (Aldh5a1(−/−) mice do not alter GHB receptor characteristics under the condition of elevated GHB levels in the brain. Membrane homogenate binding and quantitative autoradiography using [3H]NCS-382 revealed no significant changes in affinity (K_d), receptor density (B_{max}), or displacement potency (IC_{50}) in various brain regions of Aldh5a1(−/−) vs. Aldh5a1(+/+) mice (103).

With the use of [3H]GHB, binding experiments also allowed the screening of new compounds as ligands of GHB receptors. NCS-382 [104,9-tetrahydro-5-hydroxy-5H-benzocyclohept-6-ylidene-)acetic acid], a synthetic structural analog of GHB was first identified as a competitive antagonist acting at the GHB binding sites (105,106). GHB and NCS-382 completely inhibited the [3H]NCS-382 binding and NCS-382 was found to be about 10 times more potent than GHB in this regard. A variety of other receptor ligands do not modify the binding, suggesting selectivity of this radioligand for the GHB receptors in rat brain (Mehta et al., 2001). In contrast, some anti-psychotics can bind to the GHB binding sites. Among them is (−)sulpiride which, at therapeutic dosages, may exert its influence on psychotic symptoms in part via an interaction with the GHB binding sites (107). Recently, the cyclohexene and cyclopentene analogs, 3-hydroxycyclohex-1-enecarboxylic acid [(*RS*)-HOCHCA] and 3-hydroxycyclopent-1-enecarboxylic acid [(*RS*)-HOCPCA], have been found to be high-affinity GHB ligands, with stereoselectivity. The best inhibitor of these novel GHB analogs, (*R*)-HOCPCA, displays an affinity 39 times higher than GHB

in inhibition of [3H]NCS-382 binding, and is thus among the best GHB ligands reported to date (104).

Based on properties of the GHB specific binding sites, native GHB receptors in the brain have been proposed (54,108). Maitre and his colleagues have recently reported the cloning of a GHB receptor from a rat hippocampal cDNA library (109). Using the high-affinity ligand NCS-400, a 512 amino acid protein has been isolated and shown a predicted secondary structure indicative of seven transmembrane-spanning regions, However, the peptidic sequence has no significant homology with any known G-protein-coupled receptors, (GPCRs) including $GABA_B$ receptors. The GHB receptor mRNA shows a brain distribution similar to that of native GHB binding sites, but with some differences, such as high density in the cerebellum where no or very few native GHB sites have been detected (54,57,98,100). Binding assays on the recombinant protein expressed in Chinese hamster ovarian (CHO) cells showed a dissociation constant (K_d) of 426 nM for GHB but not NCS-382. Patch-clamp recordings of neurons display irreversible activation. Application of 0.1–15 μM GHB specifically induced an inward current in the transfected CHO cells that was not reproduced by application of baclofen (10 μM). The GHB receptor antagonist NCS-382 did not inhibit the GHB-induced response nor did the $GABA_B$ receptor antagonist CGP-55845. Taken together, with the presence of three mRNA bands, these authors suggested multiple subtypes of the GHB receptor (109).

Despite the convincing evidence demonstrating the presence of highly specific binding sites for GHB, the signal transduction pathway and its functional consequence following GHB binding to the putative GHB receptor are still not clear. The binding sites have been shown to be G protein-coupled receptors of the G_i or G_0 family (110). Intracellular Ca^{2+}, cyclic guanosine monophosphate (cGMP) and inositol phosphate concentrations rise after GHB administration in certain brain regions (111). For example, GHB receptor stimulation causes an increase, up to 123%, of cGMP in the hippocampus 45 min after GHB administration (500 mg/kg, i.p.) (111). The stimulation of GHB receptors by GHB induces a progressive decrease in nitric oxide synthase (NOS) activity; this reduced NOS activity may explain the GHB-induced increase in cGMP levels; other GHB receptor agonists reproduce this effect. The increase of cyclic GMP and inositol phosphates is blocked by some anticonvulsants and opiate antagonists (112), and the GHB binding site antagonist NCS-382 (105). GHB has also been shown to induce a G protein-mediated decrease in adenylyl cyclase via the presynaptic GHB receptor; this effect is blocked by a GHB antagonist (113).

In electrophysiological studies in vitro, application of GHB (25 – 50 μM) inhibits low threshold voltage-activated, T-type Ca^{2+} currents (using Ba^{2+} as the charge carrier) by 27% in differentiated neurohybridom NCB-20 cells. This GHB effect is blocked by NCS-382, but not the $GABA_B$ antagonist CGP 55845, suggesting that the modulation of the Ca^{2+} currents by GHB is mediated via the GHB receptor (114). Although the high threshold voltage-activated Ca^{2+} current is also present in these cells, any differential effect of GHB on these two main types of Ca^{2+} currents was not reported.

Yet, much remains unclear regarding the role of the GHB receptor in mediating endogenous GHB effects and the contribution of the GHB receptor-mediated response on the behavioral effects associated with supra physiologic brain concentrations induced by exogenous GHB administration.

B. $GABA_B$ Receptor Activation by GHB

The possible interaction between GHB and $GABA_B$ receptors was initially implied in the pharmacological studies on GHB in vivo. Waldmeier demonstrated that both baclofen (5–50 mg/kg i.p.) and GHB (300–1500 mg/kg i.p.) administration lead to dose-dependent increases in the striatal levels of endogenous 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) by up to 200% (116). The new $GABA_B$ antagonist, CGP 35348 (3-aminopropane-diethoxymethylphosphinic acid), blocks GHB- or baclofen-induced increase in dopamine synthesis in rat striatum, suggesting an interaction of GHB with $GABA_B$ receptors (117). Furthermore, both human absence seizures and animal experimental absence seizures could be exacerbated by direct and indirect overactivation of the GABA system. Sneed further assessed the effect of a $GABA_B$ agonist, baclofen and a specific $GABA_B$ antagonist e.g. CGP35348 in two pharmacological models of absence seizures in rodents using either GHB or pentylenetetrazole. Baclofen markedly prolonged, while the $GABA_B$ antagonist attenuated or blocked, the experimental absence seizures in both models. These data suggest a role for $GABA_B$-related mechanisms in the pathogenesis of generalized absence seizures in rodents (115).

Following these observations, Xie and Smart (1992) hypothesized that GHB-induced absence seizures could be through a direct activation of the $GABA_B$ receptor (118,119). The cellular action of GHB was studied in adult rat hippocampal neurons using intracellular recording techniques. Bath application of GHB at $< 100\ \mu M$ does not produce any detectable effects, while at 0.25 – 10 mM causes concentration-dependent hyperpolarizations under current-clamp mode or outward current when switched to voltage-clamped recordings in all hippocampal CA1 neurons studied ($n = 28$, Fig. 2A). The membrane response is associated with an increase in membrane conductance. These effects of GHB are insensitive to bicuculline and picrotoxin (both 50 μM) indicating they are not mediated by $GABA_A$ receptors, but reversibly blocked by the selective $GABA_B$ receptor antagonists, CGP 35348 or CGP 36742, suggesting that GHB directly activates $GABA_B$ receptors. Furthermore, GHB-induced responses are persistent in the presence of tetrodotoxin (1 μM), but blocked by Ba^{2+} (0.5 – 1 mM) and Zn^{2+} (300 μM), indicating a direct postsynaptic action of GHB linked to opening of a K^+ channels (Fig. 2). GHB also decreases cell excitability in response to a steady depolarizing current pulse injection (Fig. 2B). Most general anesthetics are capable of blocking voltage-gated Na^+ channels, in addition to their effect of augmenting $GABA_A$ function, and hence reduce Na^+ spikes (120). Xie investigated the possibility by examining the effects of GHB on the recombinant brain type IIa Na^+ channel (Nav1.2) expressed in Chinese hamster ovary cells in which the $GABA_B$ receptor and its coupled K^+ channels are absent. GHB at 10 mM did not inhibit the Na^+ current [unpublished observation, cf (121)].

Xie and Smart (1992b) were also the first to show a depression of excitatory and inhibitory postsynaptic potentials (EPSP-IPSP) by GHB through the activation of the $GABA_B$ receptor [119]. Orthodromic stimulation of the Schaffcr-collateral commissural pathway in the presence of bicuculline (20 μM) typically produced an EPSP followed by a slow $GABA_B$-mediated lPSP in CAI neurons (Fig. 3). GHB (1–10 mM) depressed the amplitude of both the EPSP and IPSP by 20–80% in a concentration-dependent manner (Fig. 3). GHB only slightly increased the postsynaptic membrane

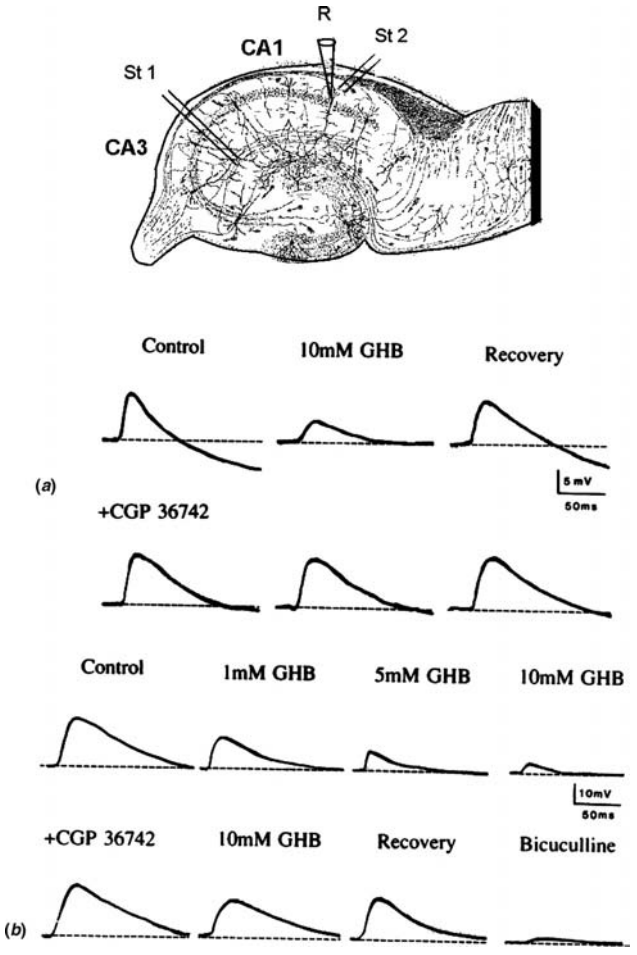


Figure 2 γ-Hydroxybutyrate (GHB) depresses excitatory post-synaptic potentials in CA 1 neurons. The EPSP and slow $GABA_B$-mediated IPSP of the recorded CA neurons (indicated as R 1 in the inserted diagram of hippocampal slice) were elicited by stimulation of Schaffer-collateral commissural pathway (15 V 40 micro s, 1 pulse every 30 s, indicated as St 1 in the inserted diagram) in 20 μM bicuculline. Bath-applied GHB at 10 mM inhibited both the EPSP and IPSP, which recovered after washing for 10 minutes in control Krebs (upper traces). CGP 36742 (500 μM) selectively blocked the slow IPSP and prevented the depression of EPSP by GHB (lower traces). Membrane potential was adjusted to −70 mV with D.C. current injections. (***b***) GHB depressed monosynaptic inhibitory postsynaptic in a different CA1 neuron. Monosynaptic IPSP were evoked by localized stimulation (10 V, 20 micro s, 1 pulse every 30 seconds, indicated as St 2 in the inserted diagram) in 20 μM CNQX. 40 μM AP5 and high magnesium (4 mM). IPSPs were concentration-dependently inhibited by 1, 5, and 10 mM GHB (upper traces). Lower traces, after washout of GHB for 10 min. the recovered IPSP was unaffected by 300 μM CGP 36742. Co-application of 10 mM of GHB and 300 μM CGP 36742 caused a small depression in IPSP. After washing CGP 36742 and GHB, subsequent application of 20 μM of bicuculline blocked the IPSP, indicating they were mediated by $GABA_A$ receptors. Membrane potential was held at − 90 mV. Traces in A and B are composed of three successive synaptic responses that were superimposed. *Source*: Adapted from Xie and Smart (1992), with permission.

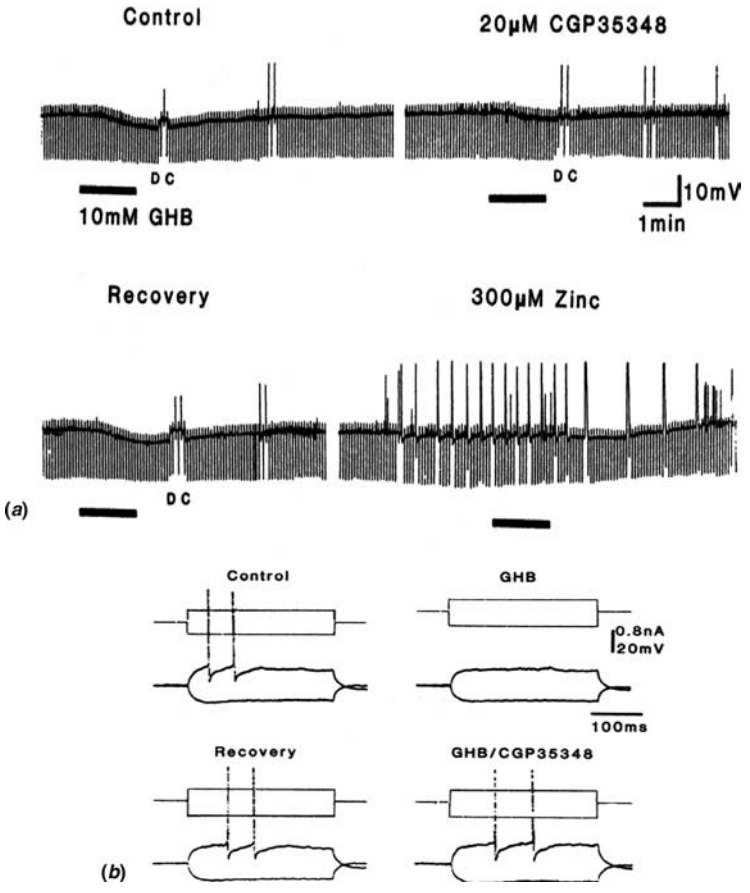


Figure 3 (***a***) Effects of CGP 35348 and zinc on the γ-hydroxybutyrate (GHB)-induccd hyperpolarization in hippocampal CA1 neumncs. (A) Upper traces are chart records of 10 mM GHB-induced responses before and during 20 μM-CGP 35348 application. Upward deflections arc action potentials evoked by depolarizing current injection (+0.4 nA, 300 ms). Downward deflections arc hyperpolarizing electrotonic potentials following current pulse injection (-0.4 nA, 300 ms, 0.2 Hz) to monitor the input conductance. Membrane potential - 60 mV adjusted with DC current injection. Lower traces from the samc cell were obtained after washout of CGP 35348 for 10 min. The GHB-induced responses were inhibited by 300 μM zinc. The upward deflections are spontaneous large depolarizing potentials indnced by zinc. In a different cell, superimposed elcctrotonic potentials (lower traces) produccd by depolarizing or hyperpolarizing current pulses (upper traces) were recordcd under coutrol conditions, during the application and subsequent recovery from GHB (10 mM) and during co-application of GHB (10 mM)+ CGP 35348 (20 μM). Resting membrane potential -62 mV. Note that in both ***a*** and ***b***, at the peak of the GHB-induced hyperpolarizing responses, the membrane potential was adjusted to the original resting level with D.C. positive current injection. Both cells were recorded using 3 M KCl-filled microelectrodes. *Source*: Adapted from Xie and Smart (1992), with permission.

conductance, which would not account for the depression of the synaptic potentials. This action of GHB was reversibly antagonized by 300–500 μM CGP 36742. The effect of GHB on the slow $GABA_B$-mediated IPSP could have resulted from a direct action on inhibitory interneurons or indirectly due to a reduction in the excitatory transmission. Therefore, Xie and Smart studied the effects of GHB on monosynaptic IPSPs evoked by localized direct stimulation of GABAergic neurons after blockade of excitatory amino acid receptors (Fig. 3). When the membrane potential was held at -90 mV with D.C. current injection, these monosynaptic IPSPs appeared to be mediated entirely by $GABA_A$ receptors as they were unaffected by CGP 36742 and completely abolished by the $GABA_A$ antagonist bicuculline (Fig. 3B). The application of GHB (l–10 mM) caused depression of the amplitude of the IPSPs by 20–80 % in a concentration-dependent fashion (Fig. 3B). The membrane potential was unaltered by GHB since it was held at the reversal potential for the GHB-induced K^+ current. In the presence of 300 μM CGP 36742, 10 mM GHB now produced only a 20–30% decrease in the amplitude of the IPSP (Fig. 3B).

These cellular actions of GHB are mimicked by baclofen, and are reversibly blocked by $GABA_B$ receptor antagonists, indicating that GHB, at much higher concentrations compared to the affinity for the GHB specific receptor, binds to and activates both pre- and postsynaptic $GABA_B$ receptors. Interestingly, the apparent antagonism of baclofen-induced responses by a low concentration of GHB (0.5 mM) suggests that GHB could act as a partial agonist at the $GABA_B$ receptors when there is a full agonist present (119). The notion of GHB as a weak partial agonist was later supported by a binding study of GHB to the $GABA_B$ binding site of the recombinant $GABA_{B(1/2)}$ receptor complex (122).

In the same period, Bernasconni and his colleagues independently found a direct interaction between GHB and $GABA_B$ using the radioligand binding assay. GHB has been demonstrated to be a selective but weak agonist for $GABA_B$ receptors. The affinity (*K*d) of GHB is approximately 150 μM (123,124). Taken together with the functional and binding studies, these findings provide convincing evidence that GHB can bind and activate $GABA_B$ receptors. A number of laboratories have since confirmed and further advanced the studies on the interaction between GHB and the $GABA_B$ receptor system on a variety of neurons in different brain regions (125–130).

Intracellular recordings of thalamocortical neurons from brain slices of the rat demonstrated that GHB (0.1–5 mM)-induced hyperpolarization and decreases in the sensory EPSP were reversibly blocked by the co-application of CGP 35348. In contrast, the GHB binding site antagonist NCS 382 did not affect the GHB-induced responses (126,127). These electrophysiological studies have demonstrated that GHB- or baclofen-induced hyperpolarization can diminish the inactivated state of T-type Ca^{2+} channels and thereby facilitate activation of low-voltage-activated Ca^{2+} channels, leading to an increase in δ sleep oscillations (126,127). The thalamic interaction between $GABA_B$-mediated hyperpolarization and T-type Ca^{2+} channel activation also underlies the generation of electroencephalographic and behavioral patterns typical of absence epilepsy under certain pathological conditions. The null succinic semialdehyde dehydrogenase (SSADH) transgenic mouse is a viable animal model for human SSADH deficiency and is characterized by markedly elevated levels of both endogenous GHB and GABA in brain, blood, and urine. The elevated endogenous GHB, similar to exogenously applied GHB, causes absence-like seizures in the transgenic mouse

which mimic the absence seizures that occurr in children with SSADH deficiency. The absence seizures in $SSADH^{(-/-)}$ mice are abolished by clinical anti-seizure drug ethosuximide and the $GABA_B$ antagonist CGP 35348 (131). The thalamic interaction between $GABA_B$ activation and T-type Ca^{2+} channel opening is confirmed by both gene knockout studies in mice. In both the alpha 1G (Cav3.1) knockout (132) and $GABA_{B(1)}$ deletion mice, neither baclofen nor GHB induce absence seizures (133). Furthermore, the alpha 1G (Cav3.1) knockout mice manifest selectively impared slow-wave sleep (134).

GHBs effect in depressing spontaneous firing rate and changing the firing pattern of dopamine neurons in the ventral tegmental area or in the substantia nigra has been found through the specific activation of $GABA_B$ receptors (129,135).

Using whole-cell patch-clamp recordings, the effects of GHB have been demonstrated to depress the frequency and amplitude of GABAergic and glutamatergic spontaneous inhibitory and excitatory post-synaptic currents (IPSCs and EPSCs) driven by presynaptic action potential firing, while the amplitude and frequency of Ca^{2+} entry-independent miniature IPSCs were not affected. All these effects of GHB were mediated via $GABA_B$ receptors (130).

Recent advances in molecular biology has revealed $GABA_B$ receptors consisting of two subunits, the $GABA_{B(1)}$ receptor and $GABA_{B(2)}$ receptor. It has been shown that $GABA_{B(2)}$ is co-expressed with $GABA_{B(1)}$ in many brain regions. The G-protein-gated inwardly rectifying potassium channels (*GIRK*) can be activated by $GABA_B$ receptor agonists only on co-expression of $GABA_{B(1)}$ with $GABA_{B(2)}$ (67,136,137). Like its effect on native $GABA_B$ receptors, GHB acts as a weak agonist at the recombinant $GABA_B$ receptors. GHB activates co-expressed $GABA_{B(1)}$ and $GABA_{B(2)}$ receptors and Kir3 channels in Xenopus oocytes with a half-maximal effective concentration (EC_{50}) of approximately 5 mM and a 70% maximal response of the effect produced by 10 μM L-baclofen. The GHB-induced current is not affected by NCS 382 (300 μM), but is completely blocked by the $GABA_B$ antagonists, CGP 35348 and CGP 54626. In the same report, using binding assay, GHB at 30 mM displaced [125I]CGP64213 binding at $GABA_{B(1)}$ expressed in COS cells by 20%. These results confirm that GHB is a weak partial agonist at the GABA binding site of $GABA_{B(1)/(2)}$ receptors (122). It is worth stressing that the equi-effective concentrations of GHB at recombinant and native $GABA_B$ receptors in binding studies differ by a factor of 100, as is the case for other $GABA_B$ agonists.

The hypocretin/orexin (Hcrt) system is a critical neurotransmitter system for the maintenance of wakefulness and the defects in this system have been linked to the cause of narcolepsy. As GHB has been used for the treatment of narcolepsy, Xie and his colleagues have recently investigated the modulation of GHB on defined Hcrt neurons using whole-cell patch-clamp recordings from transgenic mice in which enhanced green fluorescent protein (EGFP) was linked to the Hcrt promoter (Hcrt/EGFP). The hypothamic brain slices were prepared from 28-42-day old mice, when the specific GHB binding sites should be developed in the brain. Bath application of GHB at 0.1 – 10 μM did not produce any detectable electrophysiological effect. In contrast, at 1–30 mM, GHB, caused hyperpolarization (3 – 10 mV), and decreases in input resistance (by 10 – 35%) and membrane excitability in a concentration dependent manner. The EC_{50} for GHB is estimated at 4.6 mM, which is consistent with the data obtained in recombinant $GABA_B$ receptors described

above. (R)-baclofen caused a similar response with an approximately 600-fold more potent effect (EC_{50} of 7.1 μM) than GHB. The GHB effects are persistent in the presence of NCS 382 (1 mM), but reversibly blocked by CGP 52432 (2 μM), indicating they are mediated by $GABA_B$ receptor activation (213). (for details see also Chapter 34 of this volume). As has been highlighted above, multiple and independent approaches have clearly demonstrated that the vast majority of exogenous GHB-elicited electrophysiological and pharmacological effects are mediated by $GABA_B$ receptor activation in the central nervous system.

In contrast to the lack of GHB binding sites in peripheral tissue, using the sensitive reverse transcriptase-polymerase chain reaction with a specific set of primers for each isoform and Southern blot analysis, Castelli et al. (138) have found that the $GABA_B$ receptor mRNAs are expressed not only throughout the brain but also in all peripheral organs examined, including the heart, spleen, lung, liver, small intestine, large intestine, kidney, stomach, adrenal, testis, ovary and urinary bladder. While peripheral $GABA_B$ functions are still not clear, the peripheral $GABA_B$ receptors could be activated by endogenous GHB, as found at millimolar concentrations (3).

C. Relationship of GHB Binding Sites and $GABA_B$ Receptors

Limited efforts have been made to elucidate the relationship or association, if any, between these two distinct binding sites for GHB. Sneed examined the affinity of $GABA_B$ agonists and antagonists for the [3H]GHB binding site, the affinity of GHB and a GHB antagonist for the [3H]$GABA_B$ binding site, and the effect of guanine nucleotides and pertussis toxin on both, using autoradiographic binding assays. He found that GHB and its antagonist, NCS 382, did not compete for [3H]$GABA_B$ binding, nor did (R)-baclofen or the [3H]$GABA_B$ antagonists, CGP 35348 or SCH 50911, compete for [3H]GHB binding; however, the $GABA_B$ agonist 3-amino-propyl-phosphinic acid (3-APPA), and the $GABA_B$ antagonists phaclofen and 2-hydroxysaclofen (2-OH saclofen) did show a weak affinity for [3H]GHB binding in frontal cortex. These results raise the possibility that the [3H]GHB binding site may be an isoform of the presynaptic $GABA_B$ receptor (139). To further investigate this hypothesis, Sneed and his colleagues used Ca^{2+}-stimulated Rb86 efflux in synaptosomes prepared from thalamus and cortex in the presence of GHB, (R)-baclofen, or $GABA_B$ antagonists, phaclofen and CGP 35348. GHB and (R)-baclofen both suppressed K^+-stimulated [45]Ca^{2+} uptake and intracellular Ca^{2+} in synaptosomes. The effects of GHB were attenuated by the GHB receptor antagonist and phaclofen while that of (R)-baclofen was attenuated by CGP 35348. These data raise the possibility that a presynaptic GHB/$GABA_B$ receptor complex might be involved in the pathogenesis of GHB-induced generalized absence seizures [140].

It appears that GHB at high concentrations (>1 mM) exerts its ubiquitous neuropharmacological effects through $GABA_B$ receptor-mediated mechanisms. At concentrations low enough to avoid activating $GABA_B$ receptors, GHB might selectively activate the GHB binding sites and the observed responses are blocked by NCS–382. For example, some opposite effects of GHB on dopamine release, observed *in vitro* or *in vivo*, were dependent on low or very high doses. However, the differential modulation of dopamine release may not be due to activation of different receptors and many other reasons may exist (see below).

Most remarkably, Kaupmann et al. (37) have provided solid molecular evidence of the critical involvement of $GABA_B$ receptors in some GHB actions, and the separation of GHB-specific binding sites and the $GABA_B$ receptor. Kaupmann et al., studied the effects of GHB in $GABA_{B(1)}-/-$ mice, which lack functional $GABA_B$ receptors. Autoradiography reveals a similar spatial distribution of [3H]GHB-binding sites in brains of $GABA_B(1)-/-$ and wild-type mice, demonstrating that GHB-binding sites are distinct from $GABA_B$-binding sites. In the presence of the $GABA_B$ receptor, positive modulator 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol GHB induced functional GTPgamma[35S] responses in brain membrane preparations from wild-type but not $GABA_{B(1)}-/-$ mice. The GTPgamma[35S] responses in wild-type mice were blocked by the $GABA_B$ antagonist CGP54626 but not by NCS-382. Altogether, these findings suggest that the GHB-induced GTPgamma[35S] responses are mediated by $GABA_B$ receptors. Following GHB or GBL application, $GABA_{B(1)}-/-$ mice showed neither the hypolocomotion, hypothermia, increase in striatal dopamine synthesis nor electroencephalogram delta-wave induction seen in wild-type mice. It, therefore, appears that all studied GHB effects are $GABA_B$ receptor dependent (37).

IV. GHB as a Neuromodulator

Growing evidence shows that GHB causes a number of diverse neuropharmacological and neurophysiological effects (35,43,45,54,69). The suggestion that GHB has a neuromodulatory role rather than just exists as a metabolite of GABA is suggested due to the fulfillment of several criteria. First, it is synthesized in neurons heterogeneously distributed throughout the CNS, stored in vesicles, released by depolarization in a Ca^{2+}-dependent manner (141–143), and undergoes reuptake into the nerve terminal (57,69,144,145). Second, specific GHB binding sites, i.e., putative GHB receptors (42) have been identified and GHBs physiological role has been proposed. Finally, pharmacological modulations of exogenous GHB have found through the direct binding and activation of $GABA_B$ receptors in the CNS (37,118,119, 122,123,126,127,130,146,147).

A. GHB on Glutamatergic and GABAergic Systems

The ability of GHB to depress the amplitude of evoked, and the frequency of spontaneous, excitatory and inhibitory postsynaptic potentials (EPSPs-IPSPs) suggests that GHB inhibits excitatory and inhibitory neurotransmission presumably by decreasing the release of glutamate and GABA (67,119,127,130,146,148). The effects of GHB on glutamate and GABA release are concentration-dependent and mediated by direct activation of $GABA_B$ receptors as they are blocked by $GABA_B$ receptor antagonists such as CGP 35348 and not by the GHB receptor antagonist NCS 382. In contrast, NCS-382 produced a concentration-dependent increase in excitatory field potential slope by extracellular recordings (67), suggesting there may be a tonic inhibition mediated by endogenous GHB acting on GHB receptors in the hippocampus.

However, in studies using microdialysis in freely moving rats and in isolated hippocampal synaptosomes, intra-hippocampal (CA1) perfusion with GHB

(10 nM-1 mM) influenced glutamate levels in a concentration-dependent manner: GHB at 100 and 500 nM increased glutamate levels; 100 and 300 μM concentrations were ineffective; whereas the highest (1 mM) concentration reduced local glutamate levels. The GHB stimulant effect was suppressed by the locally co-perfused GHB receptor antagonist NCS-382 (10 μM) but not by the GABA(B) receptor antagonist CGP 35348 (500 μM). Furthermore, the GHB (1 mM)-induced reduction in CA1 glutamate levels was also counteracted by NCS-382 (10 μM); however, it was reversed into an increase by CGP-35348. Given alone, neither NCS-382 nor CGP-35348 modified glutamate levels *in vivo*. In hippocampal synaptosomes, GHB (50 and 100 nM) enhanced both the spontaneous and K^+-evoked glutamate efflux, both effects being blocked by NCS-382 (100 nM), but not by CGP-35348 (100 μM) [148]. In the same laboratory, using rat hippocampal slice preparations, perfusion of 500 nM and 1 mM GHB onto the slice increased and decreased extracellular glutamate levels, respectively. GHB stimulation was suppressed by NCS-382, while GHB inhibition by CGP 35348. Taken together, these findings indicate that GHB exerts a concentration-dependent biphasic regulation of hippocampal glutamate transmission via two opposing mechanisms, a direct GHB receptor-mediated facilitation at nanomolar GHB concentrations, and an $GABA_B$ receptor-mediated inhibition at millimolar concentrations (148,149).

In a separate microdialysis study, the basal and K^+-evoked release of GABA and glutamate in the superficial laminae of the frontal cortex was determined during GHB-induced absence seizures. Both the basal and K^+-evoked release of GABA were significantly decreased in laminae I-III of frontal cortex at the onset of GHB-induced absence seizures. In contrast, neither the basal nor the K^+-evoked release of glutamate was altered in superficial laminae of cerebral cortex at any time during the absence seizures. Intracortical perfusion of the $GABA_B$ receptor antagonists, CGP 35348 and phaclofen as well as the GHB receptor antagonist, NCS 382 attenuated GHB-mediated changes in the basal and K^+-evoked release of GABA. These results suggest that GHB induces a selective decrease in the release of GABA in cerebral cortex mediated by both $GABA_B$ and GHB specific receptors *in vivo* (150).

B. GHB on the Dopaminergic System

Exogenous administration of GHB raises levels of GHB many times higher than endogenous concentrations in dopaminergic regions of the brain (151,152). It appears possible that the GHBergic system participates physiologically in the control of the dopaminergic system in the nigro-striatal and in the meso-cortico-limbic pathways (153,154). Research has shown that GHB has multiple effects on dopaminergic neurons. Initially, at certain concentrations, less dopamine is released under the influence of GHB as GHB reduces impulse flow and inhibits firing of dopaminergic terminals (155,156). Decreased dopamine release is also seen with administration of baclofen, a $GABA_B$ agonist (116,125,154,157–159). It is possible that the effects of GHB on dopaminergic neurons are mediated via the GHB and/or $GABA_B$ receptor(s). With GHB, the decrease in dopamine release is followed by tissue accumulation of dopamine in the neuron (160–166). By inhibiting impulse flow, administration of GHB stimulates the kinetic properties of tyrosine hydroxylase activity; the rate limiting enzyme controlling the synthesis of dopamine from tyrosine (167–172). Histofluorescent analysis of different catecholamine systems has shown that dopamine

fibers appear swollen and abnormal suggesting an altered metabolism resulting from GHB administration (173). It appears that GHB affects dopaminergic neurons by inhibiting dopamine release; by increasing dopamine synthesis, and by inhibiting end-product inhibition of the enzyme tyrosine hydroxylase; both mechanisms increasing the amount of available dopamine in the neurons prior to GHB washout (174). Subsequent to the GHB-mediated decrease in dopamine release and increase in intercellular dopamine synthesis, a transient increase in dopamine release is observed during washout of GHB (175,176). Pretreatment with a GHB-receptor antagonist NCS 382 prevents the increase in extracellular dopamine induced by GHB. Therefore, in a similar way GHB may be affecting dopaminergic neurons via GHB and $GABA_B$ receptors (154,157,159,177). These neurons could be enkephalinergic (99) and controlled in part via μ-opioid receptors as naloxone has been reported to block the GHB-induced increase in dopamine synthesis in striatum (178).

GHB has a biphasic effect on the release of dopamine: an initial decrease in the release of transmitter was followed by an increased release. A time-dependent biphasic effect was observed when GHB was perfused onto brain slices, and a dose-dependent biphasic effect was seen in dialysate after systemic administration of GHB. Naloxone blocked GHB-induced dopamine accumulation and release both in vitro and in vivo. These data suggest that GHB influences dopamine release via GHB specific receptors that may modulate the activity of opioid interneurons. Interestingly, in a clinical case report, the anti-reward effect of the μ-opioid receptor antagonist Naltrexone likely results from its interference with the GHB-induced dopamine release, leading to a partial blockade of the GHB reinforcing effect possibly responsible for a potential craving for the drug (179).

However, there are a number of controversial reports in the effects of GHB on dopamine release. Using microdialysis in awake and urethane-anesthetized rats, the effect of GHB on striatal dopamine (DA) release was monitored for 2 hours (180). Howard and Feigenbaum found GHB (500 mg/kg, i.p.) significantly inhibited striatal DA release in conscious animals, whereas anesthetic pretreatment completely abolished the inhibitory effect of GHB on DA release.

Using different approaches, the actions of GHB on dopaminergic neuronal activity in the rat substantia nigra (SN) were studied using extracellular single unit recording techniques. Low doses ($<$ 200 mg/kg, i.p.) of GHB produced a slight excitation of the DA neurons, concomitant with a regularized firing rhythm and lack of burst activity. In higher doses, GHB produced an even higher degree of regularization, but, in contrast to low doses, an inhibition of firing rate. Administration of the $GABA_B$ receptor agonist baclofen, in all essential respects, mimicked the effect of GHB on the firing of nigral DA neurons. Both the regularization of the firing pattern and inhibition of firing rate produced by GHB are antagonized by the $GABA_B$-receptor antagonist CGP 35348 (200 mg/kg, i.v.), confirming that these actions of GHB are mediated via activation of $GABA_B$ receptors (125).

Using the same single unit recordings in vivo from urethane-anesthetized rats, the effects of GHB on evoked firing in the nucleus accumbens (NAc) "shell" neurons and on spontaneous activity of antidromically identified DA cells located in the ventral tegmental area were characterized. GHB exerted heterogeneous effects, which were correlated to the baseline firing rate of the cells but led to a moderate stimulation of

the DA system. All GHB actions were mediated by $GABA_B$ receptors. It is concluded that addictive and rewarding properties of GHB do not necessarily involve a putative high affinity GHB receptor (181).

GHB-induced bi-directional modulation of dopamine release has been linked to a differential coupling efficacy (indicated by the EC_{50} value) of G-protein-gated inwardly rectifying potassium (GIRK, Kir3) channels to $GABA_B$ receptor between dopamine neurons (requiring higher EC_{50} of $GABA_B$ receptor agonists) and GABA interneurons (lower EC_{50}) in the ventral tegmental area (VTA) (182). The coupling efficacy of G-protein-gated inwardly rectifying potassium channels to $GABA_B$ receptor was much higher in dopamine neurons than in GABA neurons depending on the differential expression of GIRK subunits (Fig. 4). Baclofen with its high affinity for the

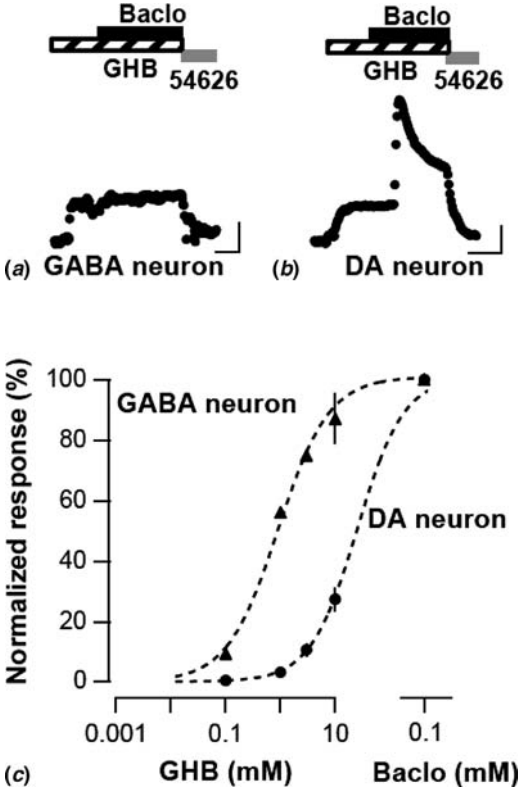


Figure 4 GHB evoked currents in the ventral tegmental area (VTA) neurons. (***a***) In a GABAergic neuron, responses to GHB (10 mM) followed by baclofen (100 μM). Scale Bars: 50 pA, 5 min. (***b***) In a dopaminergic neuron, responses to GHB (10 mM) followed by baclofen (100 μM). Note, 10 mM GHB is not saturating. Scale bars: 100 pA, 5 min. (***c***) GHB-concentration response curve normalized to the response of a saturating dose of baclofen (100 microM) in dopamine neurons (circle estimated $EC_{50} = 26.8 \pm 1.2$ mM, $h = 0.9 \pm 0.1$, $n = 4$) and GABA neurons (triangle; $EC_{50} = 0.9 \pm 0.1$ mM, $h = 1 \pm 0.1$). *Source*: Adapted from Cruz et al. (2004), with permission.

$GABA_B$ receptor would at typical therapeutic doses inhibit both GABAergic and dopaminergic neurons and overall decrease dopamine release. Conversely, GHB with low-affinity for the $GABA_B$ receptor, would, at typical recreational use concentrations (< 1 mM), preferentially inhibit GABAergic neurons and thereby increase dopamine release (183). Furthermore, bi-directional modulation of DA neurons by baclofen is dependent of its concentrations. At 0.1 μM, baclofen increases the firing frequency, whereas higher doses (0.5 and 100 μM) decrease and eventually block all action potentials (Fig. 5). The bi-directional modulation and differential coupling of $GABA_B$ receptors to GIRK channels, therefore, explains long-standing controversy over whether GHB inhibits or stimulates the dopamine system and reconciles the paradoxical finding that GHB has anti-craving effects and in some situations also has abuse liability (Fig. 5) (35,184).

C. GHB on the noradrenergic system

Anatomical, neurochemical, and electrophysiological studies have provided evidence that $GABA_B$ receptors are involved in the regulation of noradrenergic neurons emanating from the locus coeruleus (LC). $GABA_B$ activation by baclofen inhibits spontaneous firing and causes membrane hyperpolarization due to an increase in K^+ conductance (185). In reports as early as 1980, GHB has been shown to affect noradrenergic transmission in the CNS. Intraperitoneal GHB increases brain norepinephrine (NE) synthesis and utilization, particularly in the neocortex (186). Recently, Szabo et al., (187) used in vivo extracellular unitary recordings to monitor sustained administration of GHB (40 mg/kg/day) on the burst firing of LC NE neurons. Two-day and 10-day continuous 24 hr GHB administrations decreased the firing activity of LC neurons $\sim$50% when compared with controls. In contrast, removal of GHB administration after 10 days of continuous 24 hour treatment, showed a 33% augmentation in LC activity for 36 hours compared to controls. In other words, chronic GHB treatment inhibits the burst firing of LC NE neurons while present in meaningful concentrations and enhances LC NE firing during drug washout and beyond. Possible inhibited LC NE activity during nighttime GHB administration may contribute to the observed sleep enhancements noted in GHB literature. Conversely, the augmentation of LC NE neurons on washout might help describe the observations of improved wakefulness and reduced cataplexy seen during the daytime after chronic nighttime administration of GHB in narcoleptic patients. Lastly, withdrawal effects have not been seen in clinical trials but have been reported in the literature regarding continuous (around-the-clock) GHB abuse (see Chapter 54 of this volume).

D. GHB on the Serotonergic System

GHB can induce an increase in serotonin turnover in striatum and in the mesolimbic areas without significantly changing absolute levels of serotonin [116,172,188,189]. This effect can be seen as 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, accumulates after GHB dosing, while K^+-induced increase in extracellular serotonin levels do not change significantly (155,156). Both baclofen (5–50 mg/kg i.p.) and GHB (300–1500 mg/kg i.p.) administration lead to dose-dependent increases in the striatal levels of endogenous 5-hydroxytryptamine (5-HT) and 5-HIAA by up to 200%. Their effects on the reduction of 5-HT release are thought to be due to cessation

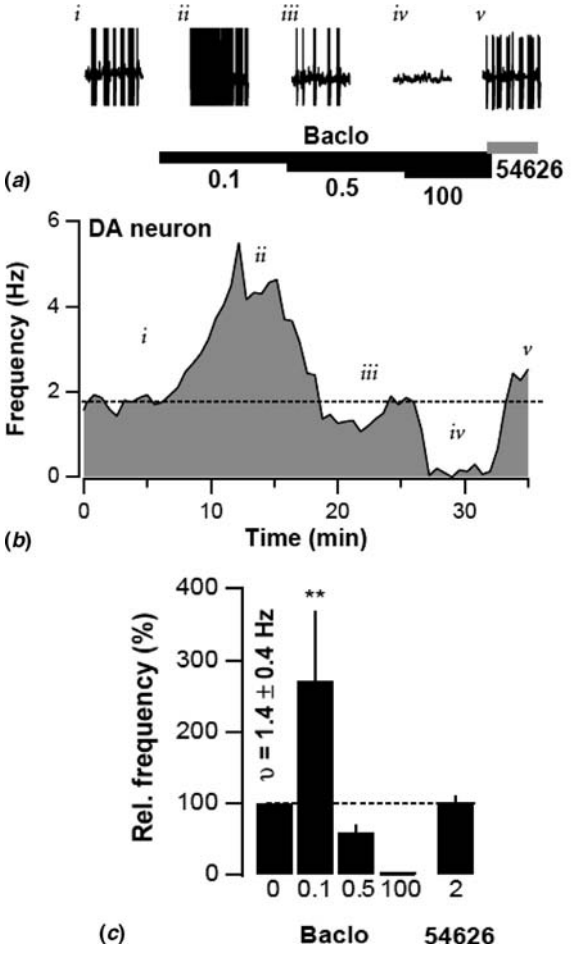


Figure 5 Bi-directional effects of $GABA_B$ receptor agonists on the firing rate of dopamine neurons in the VTA. (***a,b***) Single spike activity (***a***) recorded in the cell-attached configuration (10 s duration) from a dopamine neuron at corresponding time points of b showing the spiking frequency as a function of different baclofen doses (in μM). Low concentrations lead to a substantial increase of the firing frequency, whereas high doses eventually suppressed all action potentials. Effects were reversed by CGP 54626 (2 μM). (***c***) Average change in firing frequency. In all experiments, excitatory inputs were blocked with kynurenic acid (2 mM). *Source*: Adapted from Cruz et al. (2004), with permission.

of impulse flow. The effects on striatal 5-HT may be related to more complex phenomena (116). Furthermore, GHB may affect transport of tryptophan, the precursor of serotonin, through the blood brain barrier and/or through the neuronal membranes as noted by tryptophan accumulation after administration of GHB in vivo (190). Baclofen-induced activation of $GABA_B$ receptors mimics some aspects of the serotonergic activity of GHB (116) suggesting the effect of GHB on serotonergic neurons may be

in part mediated by GHB-induced activation of $GABA_B$ receptors; the role of GHB receptors on serotonergic activity is unclear. This action on serotonin may account for GHB's ability to stimulate growth hormone release, as coadministration with the serotonin receptor antagonist, metergoline, significantly reduces this increase (191). Metergoline has also been shown to lower GH levels in acromegaly patients (192).

E. GHB on the Opioid System

GHB has been found to increase brain levels of the endogenous opiods dynorphin and enkephalin (193,194). After a single dose of GHB (500 mg/kg, i.p.), in whole dorsal striatum, but not in other areas such as the nucleus accumbens, an increase was found in proenkephalin mRNA levels (+60%, $p < 0.01$) between 15 and 90 min after injection. An increase in prodynorphin mRNA expression was observed in the frontal cortex (+90%, $p < 0.05$) and hippocampus (+55%, $p < 0.05$) 6 h after GHB administration (112). Chronic exposure to GHB (500 mg/kg i.p. twice a day) for 10 days induced significant increases in both proenkephalin and prodynorphin mRNA levels in various brain regions (194,195).

GHB appears not to act as an agonist at the mu, delta, and kappa-opioid receptors (196). The mu-opiate antagonist naloxone (10 mg/kg) abolishes the increase of cGMP and inositol phosphate turnover in response to GHB (111), and antagonizes the decrease in brain glucose utilization induced by GHB (197). In contrast, naloxone has no effect on GHB-induced sleep and changes in dopamine metabolism (178,198,199). It should be noted that naloxone can antagonize GABA receptors (200) and GHB receptor antagonists block the GHB-induced accumulation of met-enkephalin and inhibition of release of met-enkephalin, which participates in the presynaptic regulation of dopamine release (199). Thus, GHB could act on opioid interneurons via GABAergic and/or GHBergic agonism.

F. GHB on the Cholinergic System

The effects of GHB administered on the cholinergic system are unclear. GHB receptors are located on cholinergic interneurons (99). Gamma-butyrolactone (GBL), a lactone precursor to GHB, has been shown to affect the cholinergic system. GBL produces a time dependent increase in acetylcholine in whole and subcortical rat brains; maximal increase occurs 15 min after administration of this pro-drug. A similar GHB-induced increase in acetylcholine is observed in the striatum; a 75% increase in the hippocampus 15 min after administration of the drug. GHB does not cause a significant change in the striatal or hippocampal levels of choline (201).

G. GHB and Growth Hormone

Effects of GHB on growth hormone (GH) have been described as early as 1970 (202). It is not certain if GHB's ability to stimulate GH is exclusively tied to its ability to promote non-REM sleep, the phase of sleep during a human's 24 hr diurnal cycle in which most GH is released, particularly in men. Parenthetically, there appears to be a linear relationship between SWS and GH secretion such that during aging, SW sleep and GH secretion decrease with the same chronology (203). Van Cauter et al., investigated GH response to GHB in normal young men. Eight healthy young men participated each in four experiments involving bedtime oral administration of placebo, 2.5, 3.0, and 3.5 g

of GHB. Polygraphic sleep recordings were performed every night, and blood samples were obtained at 15-min intervals from 2000 to 0800. There was a doubling of GH secretion, resulting from an increase of the amplitude and the duration of the first GH pulse after sleep onset. This stimulation of GH secretion was significantly correlated to a simultaneous increase in the amount of stage IV non-REM sleep (204). Interestingly, many of the studies looking at GHB's ability to stimulate growth hormone release were performed during daytime without causing the study participant to sleep. This suggests that GHB's ability to stimulate GH is not dependent on sleep induction.

Some of the studies evaluating GHB effect on GH in the absence of sleep are notable. Gerra et al., investigated GH responses to GHB with or without the benzodiazepine receptor antagonist, flumazenil. Nine male healthy volunteers (aged 23.2 $\pm$ 2.5 years) were submitted to three tests in random order: (1) oral GHB administration; (2) oral GHB and i.v. flumazenil administration; and (3) oral placebo and i.v. saline administration. Blood samples for GH assays were collected during the three tests at -15, 0, 15, 30, 45, 60 and 90 min. GHB induced a significant increase in GH plasma levels; flumazenil pretreatment antagonized GHB action on GH secretion. No changes were obtained with placebo and saline administration (205). The observation that baclofen stimulates GH secretion in normal men, but not in parkinsonian patients, stimulated research to test GHB's GH releasing effects in these patients. GHB, like baclofen, is a $GABA_B$ receptor agonist. $GABA_B$ activation has been shown to have a positive effect on growth hormone releasing hormone (GHRH), which in turn has a positive effect on release of GH (206). In this study, 10 normal men and 10 de novo parkinsonian patients were tested with sodium valproate (800 mg, p.o.), GHB (25 mg/kg body weight, p.o.) and baclofen (10 mg, p.o.). All drugs induced a significant increment in serum GH levels in the normal controls. On the other hand, GH secretion in parkinsonian patients did not change after baclofen or sodium valproate administration, whereas normal responsiveness to GHB was observed suggesting a different mechanism underlying the GH response to GHB (32). Volpi et al., administered GHB to normal and parkinsonian patients to investigate if muscarinic cholinergic receptors mediate the GH secretion induced by GHB; both study groups were tested in the absence and in the presence of the anticholinergic agent, pirenzepine. Both normal controls and parkinsonian patients showed a significant serum GH rise in response to GHB (25 mg/kg body weight, p.o.) although a slightly, but significantly lower response was observed in parkinsonian patients. Pretreatment with pirenzepine (100 mg p.o. 2 h before GHB) completely suppressed the GHB-induced GH release in both normal controls and parkinsonian patients. These data indicate that a cholinergic mechanism mediates the GH response to GHB in normal men and is preserved in the parkinsonian brain (207).

V. Relevance to Narcolepsy

In human narcolepsy, nightly oral administration of GHB, in doses of 4.5 g to 9 g demonstrates a dose-dependent response on sleep (increased slow-wave sleep and delta power, reduced number of awakenings), daytime sleepiness (improvement in objective and subjective measures of daytime sleepiness) and cataplexy (reduction in number of weekly cataplexy attacks). The central nervous system effect of GHB in yielding improvement of these symptoms is unknown.

To explore the potential mechanisms whereby GHB may impart beneficial effects in narcolepsy, one must first attempt to estimate brain concentrations of GHB following orally administered GHB at therapeutic doses. This is critical because of the affinity of GHB for specific GHB receptors or binding sites at low micromolar concentrations and the binding potential to $GABA_B$ receptors at millimolar concentrations, as well as the bi-directional modulation of neurotransmitter release that is dependent on the GHB concentrations interacting with a variety of neuronal circuits or cell types in many different brain areas.

A. GHB Pharmacokinetics in Humans

Pharmacokinetic profiles of GHB have been characterized in healthy volunteers, narcoleptic, alcohol dependent and GHB addict subjects after single and repeated therapeutic or recreational oral doses (15,208–211) and are summarized in Table 1. Plasma, urine, and oral fluid specimens have been frequently analyzed using gas chromatography-mass spectrometry (GC-MS). Although the data are drawn from these limited samples, the results indicated consistent time to maximum concentrations (t_{max}) that ranged between 20–50 minutes and a plasma half-life ($t_{1/2}$) of 30–50 min. Plasma maximum concentrations (C_{max}) ranged from 0.3 to 0.8 mM and were proportional to oral dosing, although the elimination kinetics of GHB is non-linear. Multiple-dose daily chronic treatment resulted neither in accumulation of GHB nor in time-dependent modification of its pharmacokinetics. At administered doses (25 – 60 mg/kg, p.o.), GHB did not accumulate in the plasma (15). The pharmacokinetics and pharmacodynamics of GHB were not changed in any clinically significant manner when the drug was chronically administered (209, 210). Food did not affect elimination and urinary excretion of unchanged drug. The only changes observed in the kinetics of GHB after 8 weeks of treatment were a 13% and 16% increase in peak concentration (C(max)) and systemic exposure (AUC), respectively (Fig. 6) (212).

The CSF kinetics of GHB in dogs with dosages of 200 to 1000 mg/kg suggests a passive diffusion of GHB into CSF with early peak brain concentrations (151). Although GHB freely crosses the BBB, the newly discovered GHB carrier-mediated transport across the BBB may further facilitate entrance of GHB into the brain (63). Assuming that the CSF kinetics of GHB in humans are similar to that in dogs with similar GHB dose and plasma concentrations in plasma, CSF and brain tissues, then following doses of 25 – 60 mg/kg (approximately 1.5 – 4.5 g, p.o. daily) brain extracellular peak concentrations would be expected to reach concentrations similar to those of the plasma (approximately 0.3 – 0.8 mM). At the typical therapeutic dose range of 4.5 to 9 g nightly for the treatment of narcolepsy, the C_{max} would be predicted to be higher than 1 mM. Again, this is purely speculative based on extrapolation from canine data, as no data exist regarding human CSF or brain concentrations following therapeutic administration of GHB.

Binding assays indicate that the affinity for GHB binding to native or recombinant $GABA_B$ receptors is approximately 120–150 μM. A number of functional studies *in vitro* and *in vivo* in animals show that the threshold concentration for the activation of $GABA_B$ receptors is around 0.25 mM. The reported EC_{50} values of GHB for $GABA_B$ receptors range from 0.8–5 mM pending on the cell type and preparation (118,119,125–130,182). It is worth emphasizing that the detection sensitivity

Table 1 Basic Pharmacokinetic Parameters for Sodium Oxybate (GHB) and Estimated Plasma Concentrations in Selected Clinical Studies

Subject	*n*	Dose (mg/kg)	Plasma concentration (C_{max} mg/L)	Plasma molar[a] (mM)	Peak time t_{max} (min)	Half-life $t_{1/2}$ (min)	Reference
Healthy	8	25	40	0.31	20–45	30	(208)
Narcoleptic	6	25–50	60	0.47	40	50	(209)
Narcoleptic	13	60	100	0.8	25–50	40	(210)
Alcohol dependent	10	25 (every 12 h for 7 days)			20–45	27	(15)
GHB addict	1	unknown	100	0.8	unknown	unknown	(211)

[a]Molar concentrations were calculated by dividing the C_{max} over the molecular weight of 126.09 g/mol of sodium oxybate (C4H7NaO3) or Xyrem®. For simplicity, the figures are rounded up.

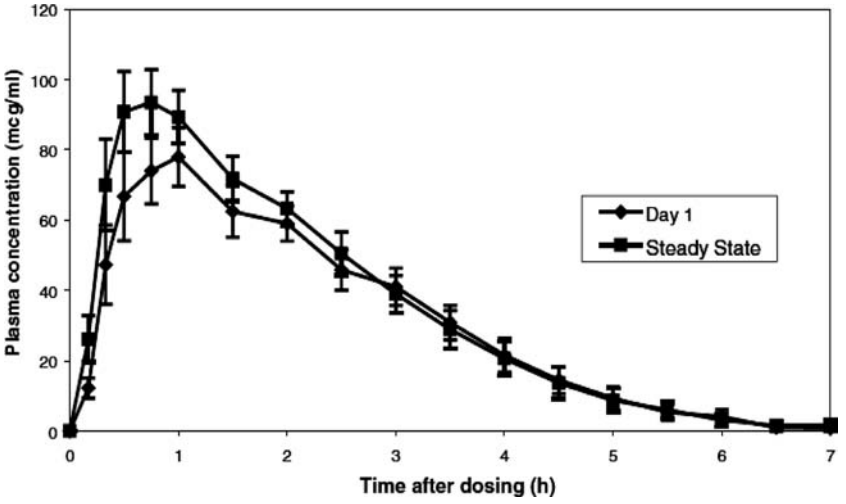


Figure 6 Mean ($\pm$SEM) plasma concentration following the initial administration of 4.5 g of sodium oxybate oral solution to narcoleptic patients and after 8 weeks of sodium oxybate therapy. Reproduced with permission from Borgen et al. (2004).

(GHB-induced hyperpolarization or outward currents and conductance changes) depends not only the applied concentration, but also on the input resistance of the recorded neurons (either as their intrinsic properties or recording quality). The higher the input resistance of the neuron, the larger the hyperpolarization (or outward current) that will occur in response to a given concentration of GHB. Both GHB and GABA are required to be present at millimolar concentrations to elicit a $GABA_B$-mediated hyperpoloarization (3–4 mV) of rat hippocampal CA1 neurons slices (118) and of mouse hypocretin neurons in hypothalamic slices (213), perhaps due to similar uptake mechanisms existing *in vitro* preparations as discussed by Madden and Johnson (129) and Crunelli and his colleagues (214). Thus, the potency of GHB for activating $GABA_B$ receptors may have been previously underestimated. Nevertheless, the resultant extracellular concentrations of GHB achieved in the brain following 4.5 – 9 g nightly dosing are predicted to attain millimolar concentrations and as such, based on a large body of animal studies, are expected to be sufficiently high to activate $GABA_B$ receptors.

B. $GABA_B$-Mediated CNS Inhibition for the Treatment of Sleep Disorders

The pioneer work of Laborit and others characterizing the sleep-promoting effects of GHB led to speculation by Mamelak that this substance may improve sleep in patients with insomnia or fragmented sleep associated with a history of psychiatric illness (33,215). GHB increase slow wave sleep (stage 3 and 4) and total sleep time in both narcoleptic patients (204,216–218,219) and healthy subjects (Van Cauter, 1997 #695). The sleep-promoting system is primarily GABAergic in nature, with the relevant cell bodies located in the ventrolateral and median preoptic area. GHB-induced

$GABA_B$-mediated hyperpolarization can promote T-type Ca^{2+} current-triggered action potential burst firing and oscillatory activity of thalamocortical neurons. This may be one of mechanisms by which GHB leads to an increase in the deep stages of sleep and provides a cellular rationale for the GHB therapy for narcolepsy (126,127).

On the other hand, the recently discovered hypocretin/orexin (Hcrt) system of the hypothalamus (220,221) has been identified as a powerful wakefulness-promoting system (222,228). Hypocretin system dysfunction occurs both in animals (223,224) and humans with narcolepsy (225–227). The Hcrt neurons have been found to receive inhibitory GABAergic input from the preoptic area (228,229). Thus, the transition from wakefulness to sleep is likely mediated by GABAergic inhibition of wake-promoting Hcrt cells, which results in disfacilitation of the wake-promoting monoaminergic and cholinergic systems.

The GABAergic system manifests both fast neurotransmission mediated through the $GABA_A$ receptors, and slower neurotransmission mediated through $GABA_B$ receptors. Xie and colleagues (213) have demonstrated that Hcrt cells are inhibited by both $GABA_A$ and $GABA_B$ receptor activation. In contrast to the transient inhibition mediated through $GABA_A$ receptors, the prolonged inhibition of Hcrt cells mediated by $GABA_B$ signaling may underlie longer-term changes in behavior and physiology in which the Hcrt cells are involved, such as the transition from wakefulness to sleep. Endogenous GHB at micromolar concentrations may activate putative GHB-specific receptors, but the functional consequences of this activity remains elusive. In contrast, exogenously administered high doses of GHB is known to activate $GABA_B$ receptors. Both clinical and basic studies suggest a role for $GABA_B$ receptors in the regulation of sleep. The $GABA_B$ agonist baclofen (25 mg) administered before sleep in a clinical study significantly prolonged total sleep time and reduced time spent awake after sleep onset (230). Exogenously administered GHB freely moves across the BBB and mimics baclofen effects on sleep modulation in humans. Many investigations have concluded that the effects of exogenously applied GHB are mediated by $GABA_B$ receptors, at least as demonstrated in preclinical models as discussed above. The recent findings that GHB and baclofen directly modulate Hcrt neuronal activity may underscore the role for $GABA_B$ receptors in sleep regulation, in addition to its broader affect of CNS inhibition. Narcolepsy is a slowly progressing neurodegenerative disorder; however, some Hcrt neurons may survive and continue to be active and exert a partial effect on the maintenance of wakefulness (225). It is possible that GHB directly and indirectly modulates the activity of remaining Hcrt neurons.

Furthermore, it has been recently reported that GHB induces a significant increase in Fos, a marker of neuronal activity, in the ventrolateral preoptic nucleus (VLPO). Although receptor mediation was not defined in this preliminary study, the ability of GHB to stimulate a sleep-promoting region of the brain may shed new light on neuronal substrates mediating its hypnotic effect (for details see also Chapter 34 in this volume) (231). In sum, GHB at high concentrations may act via $GABA_B$ receptors to consolidate sleep (reduce sleep fragmentation) and improve sleep quality (increase slow-wave sleep and delta power) at night. As a result, GHB may reduce accumulated sleep drive leading to the reduction of excessive daytime sleepness. Additionally, possible secondary daytime activation of Hcrt neurons may further improve daytime alertness. Other mechanisms may include GHB modulation of monoamine systems that may also impact sleep and wake states.

C. Mechanisms for the Control of Cataplexy

In addition to improving nocturnal sleep and daytime alertness in narcolepsy, GHB ameliorates the cataplexy of narcolepsy. Sodium oxybate (Xyrem®), was originally approved by the FDA in the United States as a drug for the treatment of cataplexy in patients with narcolepsy in July 2002 (see also chapter 54 for more details) (232). While the mechanism of action of GHB in the treatment of cataplexy associated with narcolepsy is not clear (35), GHB has been proven to be an effective agent. The question of whether a common mechanism underlying the multiple actions of GHB exists is of great research interest. GHB at micromolar concentrations, which is high enough to stimulate GHB receptors and too low to activate $GABA_B$ receptors, does not stimulate the hypocretin system in the brain (Xie, unpublished observation). However, at millimolar concentrations, GHB causes a concentration-dependent inhibition of hypocretin neuronal activity via the activation of pre and post-synaptic $GABA_B$ receptors (213). It is not clear how $GABA_B$-mediated inhibition of hypocretin neurons and other types of neurons is associated with GHBs effect in improving cataplexy. While the fundamental pathophysiology of cataplexy is poorly understood, it is thought that the norepinephrine (NE) neurons in the locus coeruleus (LC) or peri-LC neurons play a critical role. It has been convincingly shown that a strong correlation exists between the cessation of LC activity and catapleptic attacks in the canine narcoleptic model, using an extracellular single-unit recording technique combined with EEG/EMG, electrooculogram (EOG), and video camera recordings. Noradrenergic cells of the LC completely cease discharge and serotonergic cells reduce discharge to NREM sleep levels; whereas, histaminergic cells maintain waking levels of activity during cataplexy. (233–235) These observations suggest separate roles for different monoaminergic cell groups in the regulation of muscle tone and environmental awareness and in the pathophysiology of narcolepsy. Interestingly, a subset of cells in the medial medulla of the narcoleptic dog have been found to discharge at high rates only during cataplexy or REM sleep. These cells are in the medullary inhibitory area and are responsible for the hyperpolarization of motoneurons, a circuit that is activated both in cataplexy and in REM sleep. The cessation of LC activity and consequent disfacilitation of motoneurons may act synergistically with activation of the medullary atonia system to produce motor inhibition and result in cataplexy (234,236,237). Under normal physiological conditions, Hcrt cells have maximal activity during exploratory behavior and in emotional states, leading to the activation of LC cells that receive strong Hcrt innervations and project directly to spinal motor neurons (238). Under pathological conditions (loss of Hcrt neurons, in human narcolepsy and in sporatic canine narcolepsy, or mutation in Hcrt receptor 2 in familial canine narcolepsy, without the Hcrt system-induced LC-cell stimulation, failure to facilitate motor neuron activity results. Thus, the active inhibition from the medullary atonia system becomes dominant.

Such imbalance in motor function was experimentally created more than three decades ago. Injection of cholinergic agonists in the medial mesopontine reticular formations of intact cats produces a loss of muscle tone without loss of consciousness that resembles cataplexy (239). Similarly, microinjection of glutamate and corticotropin-releasing factor in the mesopontine region also induces atonia (240,241). Conversely, one would predict that GHB-induced inhibition of these "atonia-on" cells in the medial

medulla, through the activation of $GABA_B$ receptors, might produce disinhibition and consequently ameliorate the cataplexy. Although this hypothesis has not be been experimentally tested, chronic GHB treatment has been shown to inhibit the burst firing of NE neurons in the LC, while enhancing NE firing during drug washout and beyond (187). It is possible that inhibition of LC NE activity occurs during nighttime GHB administration contributing to the observed sleep enhancements. Conversely, the augmentation of LC NE neuronal activity during and after GHB washout, presumably during daytime, may not only impact improvements in wakefulness but also reduce daytime cataplexy. A limitation of this hypothesis is the latency of many weeks to months following the initiation of GHB therapy prior to achieving a maximal anti-cataplexy effect.

To further explore this hypothesis, an experiment was carried out using the immediate early gene, Fos, as a functional neuroanatomic marker of cell activity. In wake-promoting-related regions, Fos expression is decreased during sleep and increased during wake. 60–90 min after acute GHB administration (300 mg/kg, i.p.) study rats were sacrificed during light cycle. Compared to controls (saline injection) the GHB-treated animals displayed a strong increase in Fos expression in the LC, while most other wake-promoting regions, including the lateral hypothalamus, were unaffected (231). How GHB increases Fos or neuronal activity in LC neurons is not known. However, the selective increased Fos activity in the LC may be related to the reduction of cataplexy in narcoleptics. Much more work is needed in the exploration of the possible mechanisms of GHB underlying its efficacy in the treatment of the multiple symptoms of narcolepsy.

VI. Conclusions

GHB is an endogenous short chain fatty acid that is primarily metabolized from GABA in the brain. GHB high-affinity binding sites or putative specific GHB receptors and their physiological functions remains elusive. In contrast, exogenous GHB, as sodium oxybate (Xyrem®) at therapeutic doses produces a variety of pharmacological effects. At the cellular level, high concentrations of GHB principally activate $GABA_B$ receptors leading to the opening of G-protein-gated inwardly rectifying potassium channels causing hyperpolarization with an increase in membrane conductance. The direct $GABA_B$-mediated inhibition of hypocretin and other wake-promoting neurons by GHB may explain its effect of preferential enhancement of non-REM sleep. Additionally, the effects of simultaneously stimulating (indicated by Fos activity) both wake-promoting regions, such as the LC, and sleep-promoting areas, such as VLPO, may provide insight into possible mechanisms of GHB underlying its alerting and anti-cataplexy effects. Much remains to be learned about the physiological role of endogenous GHB and about the neuro-pharmacological effects of exogenous GHB.

Acknowledgments

Work for this review was supported in part by NIH R43 MH07162, and R44 RR017182 to Dr. Xie. The authors wish to express much gratitude to Anna Lopez for her dedicated clerical assistance in the preparation of this chapter.

References

1. Roth, R.H., Formation and regional distribution of gamma-hydroxybutyric acid in mammalian brain. Biochem Pharmacol, 1970; 19(12):3013–3019.
2. Ehrhardt, J.D., P. Vayer, and M. Maitre, A rapid and sensitive method for the determination of gamma-hydroxybutyric acid and trans-gamma-hydroxycrotonic acid in rat brain tissue by gas chromatography/mass spectrometry with negative ion detection. Biomed Environ Mass Spectrom, 1988; 15(10):521–524.
3. Nelson, T., et al., The extraneural distribution of gamma-hydroxybutyrate. J Neurochem, 1981; 37(5):1345–1348.
4. Strong, A.J., gamma-Hydroxybutyric acid and intracranial pressure. Lancet, 1984; 1(8389):1304.
5. Miller, S.L. and G. Schlesinger, Prebiotic syntheses of vitamin coenzymes: II. Pantoic acid, pantothenic acid, and the composition of coenzyme A. J Mol Evol, 1993; 36(4):308–314.
6. Kolin, A., et al., Cardioprotective action of sodium gamma-hydroxybutyrate against isoproterenol induced myocardial damage. Int J Exp Pathol, 1993; 74(3):275–281.
7. Laborit, G., et al., [Value of sodium gamma hydroxybutyrate in cerebral protection during severe hypoxia in the rat]. Agressologie, 1980; 21(4):189–197.
8. Artru, A.A., P.A. Steen, and J.D. Michenfelder, gamma-Hydroxybutyrate: cerebral metabolic, vascular, and protective effects. J Neurochem, 1980; 35(5):1114–1119.
9. Malyshev, V.V., L.I. Treshchuk, and N.G. Belykh, [Evaluation of the protective effect of gamma-hydroxybutyric acid in stress]. Biull Eksp Biol Med, 1981; 91(5):537–539.
10. Mamelak, M. and P. Webster, Treatment of narcolepsy and sleep apnea with gammahydroxybutyrate: a clinical and polysomnographic case study. Sleep, 1981; 4(1):105–111.
11. Ueki, Y., [Effects of gamma-hydroxybutyrate on monoamine metabolism and protein synthesis after transient global cerebral ischemia]. No Shinkei Geka, 1992; 20(9):937–946.
12. Geldenhuys, F.G., E.W. Sonnendecker, and M.C. De Klrk, Experience with sodium-gamma-4-hydroxybutyric acid (gamma-OH) in obstetrics. J Obstet Gynaecol Br Commonw, 1968; 75(4):405–413.
13. Ferrara, S.D., et al., Effects of single dose of gamma-hydroxybutyric acid and lorazepam on psychomotor performance and subjective feelings in healthy volunteers. Eur J Clin Pharmacol, 1999; 54(11):821–827.
14. Addolorato, G., et al., An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. GHB Study Group. Alcohol Alcohol, 1996; 31(4):341–345.
15. Ferrara, S.D., et al., Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. Br J Clin Pharmacol, 1992; 34(3):231–235.
16. Gallimberti, L., et al., gamma-Hydroxybutyric acid in the treatment of alcohol dependence: a double-blind study. Alcohol Clin Exp Res, 1992; 16(4):673–676.
17. Biggio, G., et al., Suppression of voluntary alcohol intake in rats and alcoholics by gamma-hydroxybutyric acid: a non-GABAergic mechanism. Adv Biochem Psychopharmacol, 1992; 47:281–288.
18. Gessa, G.L. and L. Gallimberti, Gamma-hydroxybutyric acid in the treatment of alcohol dependence. Clin Neuropharmacol, 1992; 15 Suppl 1 Pt A: 303A–304A.
19. Gallimberti, L., et al., Gamma-hydroxybutyric acid for treatment of alcohol withdrawal syndrome. Lancet, 1989; 2(8666):787–789.
20. Maremmani, I., F. Lamanna, and A. Tagliamonte, Long-term therapy using GHB (sodium gamma hydroxybutyrate) for treatment-resistant chronic alcoholics. J Psychoactive Drugs, 2001; 33(2):135–142.
21. Nimmerrichter, A.A., et al., Double-blind controlled trial of gamma-hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. Alcohol Alcohol, 2002; 37(1):67–73.
22. Bowles, T.M., R.W. Sommi, and M. Amiri, Successful management of prolonged gamma-hydroxybutyrate and alcohol withdrawal. Pharmacotherapy, 2001; 21(2):254–257.
23. Gallimberti, L., et al., Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. Alcohol, 2000; 20(3):257–262.
24. Gallimberti, L., et al., Gamma-hydroxybutyric acid for treatment of opiate withdrawal syndrome. Neuropsychopharmacology, 1993; 9(1):77–81.
25. Scharf, M.B., M. Baumann, and D.V. Berkowitz, The effects of sodium oxybate on clinical symptoms and sleep patterns in patients with fibromyalgia. J Rheumatol, 2003; 30(5):1070–1074.

26. Scharf, M.B., et al., Effect of gamma-hydroxybutyrate on pain, fatigue, and the alpha sleep anomaly in patients with fibromyalgia. Preliminary report. J Rheumatol, 1998; 25(10):1986–1990.
27. Rinaldi, F., et al., [On the use of gamma-hydroxybutyrate of sodium in psychiatric therapy]. Acta Neurol (Napoli), 1967; 22(1):21–41.
28. Martellotta, M.C., et al., Gamma-hydroxybutyric acid decreases intravenous cocaine self-administration in rats. Pharmacol Biochem Behav, 1998; 59(3):697–702.
29. Lapierre, O., et al., The effect of gamma-hydroxybutyrate on nocturnal and diurnal sleep of normal subjects: further considerations on REM sleep-triggering mechanisms. Sleep, 1990; 13(1):24–30.
30. Mamelak, M., J.M. Escriu, and O. Stokan, Sleep-inducing effects of gammahydroxybutyrate. Lancet, 1973; 2(7824):328–329.
31. Ferrari, M., et al., [Pharmacologico-clinical trials of the association of L-dopa and gamma-hydroxybutyric acid in therapy of Parkinson's disease]. Clin Ter, 1971; 57(4):351–356.
32. Volpi, R., et al., Different control mechanisms of growth hormone (GH) secretion between gamma-amino- and gamma-hydroxy-butyric acid: neuroendocrine evidence in Parkinson's disease. Psychoneuroendocrinology, 1997; 22(7):531–538.
33. Mamelak, M., Neurodegeneration, sleep, and cerebral energy metabolism: a testable hypothesis. J Geriatr Psychiatry Neurol, 1997; 10(1):29–32.
34. Abanades, S., A.M. Peiro, and M. Farre, [Club drugs: old medicines as new party drugs]. Med Clin (Barc), 2004; 123(8):305–311.
35. Maitre, M., et al., [A mechanism for gamma-hydroxybutyrate (GHB) as a drug and a substance of abuse]. Med Sci (Paris), 2005; 21(3):284–289.
36. Anderson, I.B., et al., Trends in gamma-hydroxybutyrate (GHB) and related drug intoxication: 1999 to 2003. Ann Emerg Med, 2006; 47(2):177–183.
37. Kaupmann, K., et al., Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. Eur J Neurosci, 2003; 18(10):2722–2730.
38. Queva, C., et al., Effects of GABA agonists on body temperature regulation in GABA(B(1))−/− mice. Br J Pharmacol, 2003; 140(2):315–322.
39. Meerlo, P., et al., Effects of gamma-hydroxybutyrate (GHB) on vigilance states and EEG in mice. Sleep, 2004; 27(5):899–904.
40. Carter, L.P., et al., Novel gamma-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and GABAB receptor agonists. J Pharmacol Exp Ther, 2005; 313(3):1314–1323.
41. Snead, O.C., 3rd, The gamma-hydroxybutyrate model of absence seizures: correlation of regional brain levels of gamma-hydroxybutyric acid and gamma-butyrolactone with spike wave discharges. Neuropharmacology, 1991; 30(2):161–167.
42. Snead, O.C., 3rd, et al., Increased gamma-hydroxybutyric acid receptors in thalamus of a genetic animal model of petit mal epilepsy. Epilepsy Res, 1990; 7(2):121–128.
43. Snead, O.C., 3rd and K.M. Gibson, Gamma-hydroxybutyric acid. N Engl J Med, 2005; 352(26):2721–2732.
44. Wong, C.G., T. Bottiglieri, and O.C. Snead, 3rd, GABA, gamma-hydroxybutyric acid, and neurological disease. Ann Neurol, 2003; 54 Suppl 6: S3–12.
45. Crunelli, V., Z. Emri, and N. Leresche, Unravelling the brain targets of gamma-hydroxybutyric acid. Curr Opin Pharmacol, 2006; 6(1):44–52.
46. Laborit, H., et al., [Generalities concerning the experimental study and clinical use of gamma hydroxybutyrate of Na.]. Agressologie, 1960; 1:397–406.
47. Laborit, H., Sodium 4-Hydroxybutyrate. Int J Neuropharmacol, 1964; 32:433–451.
48. Bessman, S.P. and W.N. Fishbein, Gamma-Hydroxybutyrate, a Normal Brain Metabolite. Nature, 1963; 200:1207–1208.
49. Gold, B.I. and R.H. Roth, Kinetics of in vivo conversion of gamma-[3H]aminobutyric acid to gamma-[3H]hydroxybutyric acid by rat brain. J Neurochem, 1977; 28(5):1069–1073.
50. Anderson, R.A., R.F. Ritzmann, and B. Tabakoff, Formation of gamma-hydroxybutyrate in brain. J Neurochem, 1977; 28(3):633–639.
51. Roth, R.H. and N.J. Giarman, Evidence that central nervous system depression by 1,4-butanediol in mediated through a metabolite, gamma-hydroxybutyrate. Biochem Pharmacol, 1968; 17(5):735–739.
52. Weissmann-Nanopoulos, D., et al., Immunocytochemical evidence for the presence of enzymes synthesizing GABA and GHB in the same neuron. Neurochem Int, 1984(6):333–338.

53. Rumigny, J.F., et al., Evidence that a specific succinic semialdehyde reductase is responsible for gamma-hydroxybutyrate synthesis in brain tissue slices. FEBS Lett, 1981; 134(1):96–98.
54. Maitre, M., The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. Prog Neurobiol, 1997; 51(3):337–361.
55. Snead, O.C., 3rd, R. Furner, and C.C. Liu, In vivo conversion of gamma-aminobutyric acid and 1,4-butanediol to gamma-hydroxybutyric acid in rat brain. Studies using stable isotopes. Biochem Pharmacol, 1989; 38(24):4375–4380.
56. Eli, M. and F. Cattabeni, Endogenous gamma-hydroxybutyrate in rat brain areas: postmortem changes and effects of drugs interfering with gamma-aminobutyric acid metabolism. J Neurochem, 1983; 41(2):524–530.
57. Benavides, J., et al., High affinity binding sites for gamma-hydroxybutyric acid in rat brain. Life Sci, 1982; 30(11):953–961.
58. Hearl, W.G. and J.E. Churchich, A mitochondrial NADP + -dependent reductase related to the 4-aminobutyrate shunt. Purification, characterization, and mechanism. J Biol Chem, 1985; 260(30):16361–16366.
59. Cho, S.W., et al., Kinetics and mechanism of an NADPH-dependent succinic semialdehyde reductase from bovine brain. Eur J Biochem, 1993; 211(3):757–762.
60. Santaniello, E., A. Manzocchi, and L. Tosi, Evaluation of gamma-hydroxybutyrate formed from L-glutamate by mouse brain homogenate. J Neurochem, 1978; 31(4):1117–1118.
61. Barker, S.A., et al., Identification and quantitation of 1,4-butanediol in mammalian tissues: an alternative biosynthetic pathway for gamma-hydroxybutyric acid. Biochem Pharmacol, 1985; 34(10):1849–1852.
62. Snead, O.C., 3rd, C.C. Liu, and L.J. Bearden, Studies on the relation of gamma-hydroxybutyric acid (GHB) to gamma-aminobutyric acid (GABA). Evidence that GABA is not the sole source for GHB in rat brain. Biochem Pharmacol, 1982; 31(23):3917–3923.
63. Bhattacharya, I. and K.M. Boje, GHB (gamma-hydroxybutyrate) carrier-mediated transport across the blood-brain barrier. J Pharmacol Exp Ther, 2004; 311(1):92–98.
64. Lettieri, J. and H.L. Fung, Improved pharmacological activity via pro-drug modification: comparative pharmacokinetics of sodium gamma-hydroxybutyrate and gamma-butyrolactone. Res Commun Chem Pathol Pharmacol, 1978; 22(1):107–118.
65. Lettieri, J.T. and H. Fung, Evaluation and development of gas chromatographic procedures for the determination of gamma-hydroxybutyric acid and gamma-butyrolactone in plasma. Biochem Med, 1978; 20(1):70–80.
66. Snead, O.C., III, Effect of gamma-crotonolactone in experimental models of generalized absence seizures. Brain Res, 1991; 543(1):10–14.
67. Bettler, B., et al., Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev, 2004; 84(3):835–867.
68. Doherty, J.D., O.C. Snead, and R.H. Roth, A sensitive method for quantitation of gamma-hydroxybutyric acid and gamma-butyrolactone in brain by electron capture gas chromatography. Anal Biochem, 1975; 69(1):268–277.
69. Snead, O.C., 3rd, Gamma hydroxybutyrate. Life Sci, 1977; 20(12):1935–1943.
70. Vayer, P., et al., Effect of anticonvulsant drugs on gamma-hydroxybutyrate release from hippocampal slices: inhibition by valproate and ethosuximide. J Neurochem, 1987; 49(4):1022–1024.
71. Doherty, J.D., et al., Identification of endogenous gamma-hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. J Pharmacol Exp Ther, 1978; 207(1):130–139.
72. Snead, O.C., 3rd, gamma-Hydroxybutyric acid in subcellular fractions of rat brain. J Neurochem, 1987; 48(1):196–201.
73. Snead, O.C., 3rd and B.J. Morley, Ontogeny of gamma-hydroxybutyric acid. I. Regional concentration in developing rat, monkey and human brain. Brain Res, 1981; 227(4):579–589.
74. Doherty, J.D., R.W. Stout, and R.H. Roth, Metabolism of (1–14C)gamma-hydroxybutyric acid by rat brain after intraventricular injection. Biochem Pharmacol, 1975; 24(4):469–474.
75. Mohler, H., A.J. Patel, and Balazs, Gamma-hydroxybutyrate degradation in the brain in vivo: negligible direct conversion to GABA. J Neurochem, 1976; 27(1):253–258.
76. Doherty, J.D. and R.H. Roth, Metabolism of gamma-hydroxy-[1–14C] butyrate by rat brain: relationship to the Krebs cycle and metabolic compartmentation of amino acids. J Neurochem, 1978; 30(6):1305–1309.

77. Vayer, P., et al., Evidence for a role of high Km aldehyde reductase in the degradation of endogenous gamma-hydroxybutyrate from rat brain. FEBS Lett, 1985; 190(1):55–60.
78. Kaufman, E.E., et al., Purification and characterization of an NADP + -linked alcohol oxido-reductase which catalyzes the interconversion of gamma-hydroxybutyrate and succinic semialdehyde. J Neurochem, 1979; 32(3):699–712.
79. Kaufman, E.E. and T. Nelson, Evidence for the participation of a cytosolic NADP + -dependent oxidoreductase in the catabolism of gamma-hydroxybutyrate in vivo. J Neurochem, 1987; 48(6):1935–1941.
80. Kaufman, E.E., N. Relkin, and T. Nelson, Regulation and properties of an NADP+ oxidoreductase which functions as a gamma-hydroxybutyrate dehydrogenase. J Neurochem, 1983; 40(6):1639–1646.
81. Weissmann-Nanopoulos, D., et al., Immunocytochemical localization in rat brain of the enzyme that synthesizes Gamma-hydroxybutyric acid. Neurochem Int, 1982(4):523–529.
82. Kaufman, E.E. and T. Nelson, Kinetics of coupled gamma-hydroxybutyrate oxidation and D-glucuronate reduction by an NADP + -dependent oxidoreductase. J Biol Chem, 1981; 256(13):6890–6894.
83. Kaufman, E.E. and T. Nelson, An overview of gamma-hydroxybutyrate catabolism: the role of the cytosolic NADP(+)-dependent oxidoreductase EC 1.1.1.19 and of a mitochondrial hydroxyacid-oxoacid transhydrogenase in the initial, rate-limiting step in this pathway. Neurochem Res, 1991; 16(9):965–974.
84. Taberner, P.V., J.T. Rick, and G.A. Kerkut, The action of gamma-hydroxybutyric acid on cerebral glucose metabolism. J Neurochem, 1972; 19(2):245–254.
85. Snead, O.C., 3rd, L.J. Bearden, and V. Pegram, Effect of acute and chronic anticonvulsant administration on endogenous gamma-hydroxybutyrate in rat brain. Neuropharmacology, 1980; 19(1):47–52.
86. Kaufman, E.E., et al., Oxidation of gamma-hydroxybutyrate to succinic semialdehyde by a mitochondrial pyridine nucleotide-independent enzyme. J Neurochem, 1988; 51(4):1079–1084.
87. Mitoma, C. and S.E. Neubauer, Gamma-hydroxybutyric acid and sleep. Experientia, 1968; 24(1):12–13.
88. Margolis, R.K., The effect of gamma-hydroxybutyric acid on amino acid levels in brain. Biochem Pharmacol, 1969; 18(5):1243–1246.
89. Vayer, P., P. Mandel, and M. Maitre, Conversion of gamma-hydroxybutyrate to gamma-aminobutyrate in vitro. J Neurochem, 1985; 45(3):810–814.
90. Vayer, P., et al., Natural occurrence of trans-gamma hydroxycrotonic acid in rat brain. Biochem Pharmacol, 1985; 34(13):2401–2404.
91. Hechler, V., C. Ratomponirina, and M. Maitre, gamma-Hydroxybutyrate conversion into GABA induces displacement of GABAB binding that is blocked by valproate and ethosuximide. J Pharmacol Exp Ther, 1997; 281(2):753–760.
92. Walkenstein, S.S., et al., Metabolism of Gamma-Hydroxybutyric Acid. Biochim Biophys Acta, 1964; 86:640–642.
93. Gibson, K.M., et al., Significant behavioral disturbances in succinic semialdehyde dehydrogenase (SSADH) deficiency (gamma-hydroxybutyric aciduria). Biol Psychiatry, 2003; 54(7):763–768.
94. Arnulf, I., et al., Effect of genetically caused excess of brain gamma-hydroxybutyric acid and GABA on sleep. Sleep, 2005; 28(4):418–424.
95. Hogema, B.M., et al., Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. Nat Genet, 2001; 29(2):212–216.
96. Gupta, M., et al., Murine succinate semialdehyde dehydrogenase deficiency. Ann Neurol, 2003; 54 Suppl 6: S81–S90.
97. Maitre, M., et al., High affinity binding site for gamma-hydroxybutyric acid in rat brain. Adv Biochem Psychopharmacol, 1983; 37:441–453.
98. Snead, O.C., 3rd and C.C. Liu, Gamma-hydroxybutyric acid binding sites in rat and human brain synaptosomal membranes. Biochem Pharmacol, 1984; 33(16):2587–2590.
99. Kawaguchi, Y., et al., Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci, 1995; 18(12):527–535.
100. Snead, O.C., 3rd, The ontogeny of [3H]gamma-hydroxybutyrate and [3H]GABAB binding sites: relation to the development of experimental absence seizures. Brain Res, 1994; 659(1–2):147–156.
101. Mamelak, M. and D. Hyndman, Gamma-Hydroxybutyrate and oxidative stress. Gamma-Hydroxybutyrate: Molecular, functional and clinical aspects., G. Tunnicliff and C. Cash, Editors. 2002. p. 218–235.
102. Sherman, I.A., F.G. Saibil, and T.I. Janossy, gamma-Hydroxybutyrate mediated protection of liver function after long-term hypothermic storage. Transplantation, 1994; 57(1):8–11.

103. Mehta, A.K., et al., Succinate semialdehyde dehydrogenase deficiency does not down-regulate gamma-hydroxybutyric acid binding sites in the mouse brain. Mol Genet Metab, 2006.
104. Wellendorph, P., et al., Novel cyclic gamma-hydroxybutyrate (GHB) analogs with high affinity and stereoselectivity of binding to GHB sites in rat brain. J Pharmacol Exp Ther, 2005; 315(1):346–351.
105. Maitre, M., et al., A specific gamma-hydroxybutyrate receptor ligand possesses both antagonistic and anticonvulsant properties. J Pharmacol Exp Ther, 1990; 255(2):657–663.
106. Castelli, M.P., et al., A review of pharmacology of NCS-382, a putative antagonist of gamma-hydroxybutyric acid (GHB) receptor. CNS Drug Rev, 2004; 10(3):243–260.
107. Maitre, M., et al., Displacement of [3H] gamma-hydroxybutyrate binding by benzamide neuroleptics and prochlorperazine but not by other antipsychotics. Eur J Pharmacol, 1994; 256(2):211–214.
108. Maitre, M., et al., Gamma-hydroxybutyric acid as a signaling molecule in brain. Alcohol, 2000; 20(3):277–283.
109. Andriamampandry, C., et al., Cloning and characterization of a rat brain receptor that binds the endogenous neuromodulator gamma-hydroxybutyrate (GHB). Faseb J, 2003; 17(12):1691–1693.
110. Ratomponirina, C., et al., gamma-Hydroxybutyrate receptor binding in rat brain is inhibited by guanyl nucleotides and pertussis toxin. Neurosci Lett, 1995; 189(1):51–53.
111. Vayer, P., et al., 3′-5′ cyclic-guanosine monophosphate increase in rat brain hippocampus after gamma-hydroxybutyrate administration. Prevention by valproate and naloxone. Life Sci, 1987; 41(5):605–610.
112. Vayer, P. and M. Maitre, Gamma-hydroxybutyrate stimulation of the formation of cyclic GMP and inositol phosphates in rat hippocampal slices. J Neurochem, 1989; 52(5):1382–1387.
113. Snead, O.C., 3rd, Evidence for a G protein-coupled gamma-hydroxybutyric acid receptor. J Neurochem, 2000; 75(5):1986–1996.
114. Kemmel, V., et al., Gamma-hydroxybutyrate receptor function determined by stimulation of rubidium and calcium movements from NCB-20 neurons. Neuroscience, 2003; 116(4):1021–1031.
115. Snead, O.C., 3rd, Evidence for GABAB-mediated mechanisms in experimental generalized absence seizures. Eur J Pharmacol, 1992; 213(3):343–349.
116. Waldmeier, P.C. and B. Fehr, Effects of baclofen and gamma-hydroxybutyrate on rat striatal and mesolimbic 5-HT metabolism. Eur J Pharmacol, 1978; 49(2):177–184.
117. Waldmeier, P.C., The GABAB antagonist, CGP 35348, antagonizes the effects of baclofen, gamma-butyrolactone and HA 966 on rat striatal dopamine synthesis. Naunyn Schmiedebergs Arch Pharmacol, 1991; 343(2):173–178.
118. Xie, X. and T.G. Smart, Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating GABAB receptors. Eur J Pharmacol, 1992; 212(2–3):291–294.
119. Xie, X. and T.G. Smart, gamma-Hydroxybutyrate depresses monosynaptic excitatory and inhibitory postsynaptic potentials in rat hippocampal slices. Eur J Pharmacol, 1992; 223(2–3):193–196.
120. Lingamaneni, R., M.L. Birch, and H.C. Hemmings, Jr., Widespread inhibition of sodium channel-dependent glutamate release from isolated nerve terminals by isoflurane and propofol. Anesthesiology, 2001; 95(6):1460–1466.
121. Xie, X., et al., Electrophysiological and pharmacological properties of the human brain type IIA Na+ channel expressed in a stable mammalian cell line. Pflugers Arch, 2001; 441(4):425–433.
122. Lingenhoehl, K., et al., Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. Neuropharmacology, 1999; 38(11):1667–1673.
123. Bernasconi, R., et al., Experimental absence seizures: potential role of gamma-hydroxybutyric acid and GABAB receptors. J Neural Transm Suppl, 1992; 35:155–177.
124. Mathivet, P., et al., Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABAB receptor agonist. Eur J Pharmacol, 1997; 321(1):67–75.
125. Engberg, G. and H. Nissbrandt, gamma-Hydroxybutyric acid (GHBA) induces pacemaker activity and inhibition of substantia nigra dopamine neurons by activating GABAB-receptors. Naunyn Schmiedebergs Arch Pharmacol, 1993; 348(5):491–497.
126. Williams, S.R., J.P. Turner, and V. Crunelli, Gamma-hydroxybutyrate promotes oscillatory activity of rat and cat thalamocortical neurons by a tonic GABAB, receptor-mediated hyperpolarization. Neuroscience, 1995; 66(1):133–141.
127. Emri, Z., K. Antal, and V. Crunelli, Gamma-hydroxybutyric acid decreases thalamic sensory excitatory postsynaptic potentials by an action on presynaptic GABAB receptors. Neurosci Lett, 1996; 216(2):121–124.

128. King, M.A., J.S. Thinschmidt, and D.W. Walker, Gammahydroxybutyrate (GHB) receptor ligand effects on evoked synaptic field potentials in CA1 of the rat hippocampal slice. J Neural Transm, 1997; 104(11–12):1177–1193.
129. Madden, T.E. and S.W. Johnson, Gamma-hydroxybutyrate is a GABAB receptor agonist that increases a potassium conductance in rat ventral tegmental dopamine neurons. J Pharmacol Exp Ther, 1998; 287(1):261–265.
130. Jensen, K. and I. Mody, GHB depresses fast excitatory and inhibitory synaptic transmission via GABA(B) receptors in mouse neocortical neurons. Cereb Cortex, 2001; 11(5):424–429.
131. Cortez, M.A., et al., Absence seizures in succinic semialdehyde dehydrogenase deficient mice: a model of juvenile absence epilepsy. Pharmacol Biochem Behav, 2004; 79(3):547–553.
132. Kim, D., et al., Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channels. Neuron, 2001; 31(1):35–45.
133. Schuler, V., et al., Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). Neuron, 2001; 31(1):47–58.
134. Anderson, M.P., et al., Thalamic Cav3.1 T-type Ca2+ channel plays a crucial role in stabilizing sleep. Proc Natl Acad Sci U S A, 2005; 102(5):1743–1748.
135. Erhardt, S., et al., Inhibition of firing rate and changes in the firing pattern of nigral dopamine neurons by gamma-hydroxybutyric acid (GHBA) are specifically induced by activation of GABA(B) receptors. Naunyn Schmiedebergs Arch Pharmacol, 1998; 357(6):611–619.
136. Galvez, T., et al., Mapping the agonist-binding site of GABAB type 1 subunit sheds light on the activation process of GABAB receptors. J Biol Chem, 2000; 275(52):41166–411674.
137. Galvez, T., et al., Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. Embo J, 2001; 20(9):2152–2159.
138. Castelli, M.P., et al., Distribution of GABA(B) receptor mRNAs in the rat brain and peripheral organs. Life Sci, 1999; 64(15):1321–1328.
139. Snead, O.C., 3rd, Relation of the [3H] gamma-hydroxybutyric acid (GHB) binding site to the gamma-aminobutyric acidB (GABAB) receptor in rat brain. Biochem Pharmacol, 1996; 52(8):1235–1243.
140. Snead, O.C., 3rd, Presynaptic GABAB-and gamma-hydroxybutyric acid-mediated mechanisms in generalized absence seizures. Neuropharmacology, 1996; 35(3):359–367.
141. Maitre, M. and P. Mandel, [Calcium-dependent liberation of gamma-hydroxybutyrate after depolarization of rat brain slices]. C R Seances Acad Sci III, 1982; 295(12):741–743.
142. Maitre, M., et al., Depolarization-evoked release of gamma-hydroxybutyrate from rat brain slices. J Neurochem, 1983; 41(1):287–290.
143. Vayer, P. and M. Maitre, Regional differences in depolarization-induced release of gamma-hydroxybutyrate from rat brain slices. Neurosci Lett, 1988; 87(1–2):99–103.
144. Benavides, J., et al., A high-affinity, Na + -dependent uptake system for gamma-hydroxybutyrate in membrane vesicles prepared from rat brain. J Neurochem, 1982; 38(6):1570–1575.
145. Hechler, V., et al., gamma-Hydroxybutyrate uptake by rat brain striatal slices. Neurochem Res, 1985; 10(3):387–396.
146. Berton, F., et al., Gamma-Hydroxybutyrate inhibits excitatory postsynaptic potentials in rat hippocampal slices. Eur J Pharmacol, 1999; 380(2–3):109–116.
147. Gervasi, N., et al., Pathway-specific action of gamma-hydroxybutyric acid in sensory thalamus and its relevance to absence seizures. J Neurosci, 2003; 23(36):11469–114678.
148. Ferraro, L., et al., gamma-Hydroxybutyrate modulation of glutamate levels in the hippocampus: an in vivo and in vitro study. J Neurochem, 2001; 78(5):929–939.
149. Castelli, M.P., et al., Selective gamma-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of gamma-hydroxybutyric acid. J Neurochem, 2003; 87(3):722–732.
150. Hu, R.Q., P.K. Banerjee, and O.C. Snead, 3rd, Regulation of gamma-aminobutyric acid (GABA) release in cerebral cortex in the gamma-hydroxybutyric acid (GHB) model of absence seizures in rat. Neuropharmacology, 2000; 39(3):427–439.
151. Shumate, J.S. and O.C. Snead, 3rd, Plasma and central nervous system kinetics of gamma-hydroxybutyrate. Res Commun Chem Pathol Pharmacol, 1979; 25(2):241–256.
152. Lettieri, J.T. and H.L. Fung, Dose-dependent pharmacokinetics and hypnotic effects of sodium gamma-hydroxybutyrate in the rat. J Pharmacol Exp Ther, 1979; 208(1):7–11.

153. Roth, R.H., J.D. Doherty, and J.R. Walters, Gamma-hydroxybutyrate: a role in the regulation of central dopaminergic neurons? Brain Res, 1980; 189(2):556–560.
154. Nissbrandt, H., A. Elverfors, and G. Engberg, Pharmacologically induced cessation of burst activity in nigral dopamine neurons: significance for the terminal dopamine efflux. Synapse, 1994; 17(4):217–224.
155. Bustos, G. and R.H. Roth, Effect of -hydroxybutyrate on the release of monoamines from the rat striatum. Br J Pharmacol, 1972; 44(4):817–820.
156. Bustos, G. and R.H. Roth, Release of monoamines from the striatum and hypothalamus: effect of -hydroxybutyrate. Br J Pharmacol, 1972; 46(1):101–115.
157. Da Prada, M. and H.H. Keller, Baclofen and gamma-hydroxybutyrate: similar effects on cerebral dopamine neurones. Life Sci, 1976; 19(8):1253–1263.
158. Kelly, P.H. and K.E. Moore, Dopamine concentrations in the rat brain following injections into the substantia nigra of baclofen, gamma-aminobutyric acid, gamma-hydroxybutyric acid, apomorphine and amphetamine. Neuropharmacology, 1978; 17(3):169–174.
159. Broxterman, H.J., et al., Cross-tolerance of dopamine metabolism to baclofen, gamma-butyrolactone and HA-966 in the striatum and olfactory tubercle of the rat. Life Sci, 1981; 28(9):973–981.
160. Gessa, G.L., et al., Selective increase of brain dopamine induced by gamma hydroxybutyrate. Life Sci, 1966(5):1921–1930.
161. Gessa, G.L., et al., Selective increase of brain dopamine induced by gamma-hydroxybutyrate: study of the mechanism of action. J Neurochem, 1968; 15(5):377–381.
162. Aghajanian, G.K. and R.H. Roth, Gamma-hydroxybutyrate-induced increase in brain dopamine: localization by fluorescence microscopy. J Pharmacol Exp Ther, 1970; 175(1):131–138.
163. Spano, P.F., et al., Stimulation of brain dopamine synthesis by gamma-hydroxybutyrate. J Neurochem, 1971; 18(10):1831–1836.
164. Pericic, D. and J.R. Walters, Dopamine in substantia nigra and cortex after gamma-butyrolactone treatment. J Pharm Pharmacol, 1976; 28(6):527–530.
165. Lundborg, P., T. Hedner, and J. Engel, Catecholamine concentration in the developing rat brain after gamma-hydroxybutyric acid. J Neurochem, 1980; 35(2):425–429.
166. Dyck, L.E. and C.W. Kazakoff, Acceleration of the biosynthesis of rat striatal dopamine by incubation and by administration of gamma-butyrolactone. J Neurosci Res, 1982; 8(1):57–65.
167. Roth, R.H. and Y. Suhr, Mechanism of the gamma-hydroxybutyrate-induced increase in brain dopamine and its relationship to "sleep". Biochem Pharmacol, 1970; 19(12):3001–3012.
168. Walters, J.R. and R.H. Roth, Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow. J Pharmacol Exp Ther, 1974; 191(1):82–91.
169. Walters, J.R. and R.H. Roth, Dopaminergic neurons - alteration in the sensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine after cessation of impulse flow. Biochem Pharmacol, 1975(25):649–654.
170. Zivkovic, B., A. Guidotti, and E. Costa, The regulation of striatal tyrosine hydroxylase. Effects of gamma hydroxybutric acid and healperidol. Naunyn Schmiedebergs Arch Pharmacol, 1975; 291(2):193–200.
171. Morgenroth, V.H., 3rd, J.R. Walters, and R.H. Roth, Dopaminergic neurons–alteration in the kinetic properties of tyrosine hydroxylase after cessation of impulse flow. Biochem Pharmacol, 1976; 25(6):655–661.
172. Hedner, T. and P. Lundborg, Effect of gammahydroxybutyric acid on catecholamine synthesis and utilization in the developing rat brain. J Neural Transm, 1982; 54(1–2):19–28.
173. Arluison, M., et al., Histofluorescence analysis of several systems of catecholaminergic nerve fibres within the rat neostriatum revealed by either restricted lesions of the substantia nigra or gamma-hydroxybutyrate. Brain Res Bull, 1982; 9(1–6):355–365.
174. Tunnicliff, G., Significance of gamma-hydroxybutyric acid in the brain. Gen Pharmacol, 1992; 23(6):1027–1034.
175. Cheramy, A., A. Nieoullon, and J. Glowinski, Stimulating effects of gamma-hydroxybutyrate on dopamine release from the caudate nucleus and the substantia nigra of the cat. J Pharmacol Exp Ther, 1977; 203(2):283–293.
176. Hechler, V., et al., Extracellular events induced by gamma-hydroxybutyrate in striatum: a microdialysis study. J Neurochem, 1991; 56(3):938–944.

177. Biggio, G., et al., Effect of muscimol, a GABA-mimetic agent, on dopamine metabolism in the mouse brain. Life Sci, 1977; 21(4):525–531.
178. Snead, O.C., 3rd and L.J. Bearden, Naloxone overcomes the dopaminergic, EEG, and behavioral effects of gamma-hydroxybutyrate. Neurology, 1980; 30(8):832–838.
179. Caputo, F., et al., Suppression of craving for gamma-hydroxybutyric acid by naltrexone administration: three case reports. Clin Neuropharmacol, 2005; 28(2):87–89.
180. Howard, S.G. and J.J. Feigenbaum, Effect of gamma-hydroxybutyrate on central dopamine release in vivo. A microdialysis study in awake and anesthetized animals. Biochem Pharmacol, 1997; 53(1):103–110.
181. Pistis, M., et al., Gamma-hydroxybutyric acid (GHB) and the mesoaccumbens reward circuit: evidence for GABA(B) receptor-mediated effects. Neuroscience, 2005; 131(2):465–474.
182. Cruz, H.G., et al., Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci, 2004; 7(2):153–159.
183. Feigenbaum, J.J. and S.G. Howard, Does gamma-hydroxybutyrate inhibit or stimulate central DA release? Int J Neurosci, 1996; 88(1–2):53–69.
184. Wong, C.G., K.M. Gibson, and O.C. Snead, 3rd, From the street to the brain: neurobiology of the recreational drug gamma-hydroxybutyric acid. Trends Pharmacol Sci, 2004; 25(1):29–34.
185. Shefner, S.A. and S.S. Osmanovic, GABAA and GABAB receptors and the ionic mechanisms mediating their effects on locus coeruleus neurons. Prog Brain Res, 1991; 88:187–195.
186. Persson, B. and M. Henning, Central cardiovascular effects of gamma-hydroxybutyric acid: interactions with noradrenaline, serotonin, dopamine and acetylcholine transmission. Acta Pharmacol Toxicol (Copenh), 1980; 47(5):335–346.
187. Szabo, S.T., et al., Effects of sustained gamma-hydroxybutyrate treatments on spontaneous and evoked firing activity of locus coeruleus norepinephrine neurons. Biol Psychiatry, 2004; 55(9):934–939.
188. Spano, P.F. and E. Przegalinski, Stimulation of serotonin synthesis by anesthetic and non-anesthetic doses of gamma-hydroxybutyrate. Pharmacol. Res. Commun., 1973(5):55–69.
189. Miguez, I., et al., Effect of low doses of gamma-hydroxybutyric acid on serotonin, noradrenaline, and dopamine concentrations in rat brain areas. Neurochem Res, 1988; 13(6):531–533.
190. Boadle-Biber, M.C., Biosynthesis of serotonin. Biology of Serotonergic Transmission, ed. N.N. Osborne. 1982, New York: Wiley.
191. Gerra, G., et al., Naloxone and metergoline effects on growth hormone response to gamma-hydroxybutyric acid. Int Clin Psychopharmacol, 1995; 10(4):245–250.
192. Delitala, G., et al., Growth hormone and prolactin release in acromegalic patients following metergoline administration. J Clin Endocrinol Metab, 1976; 43(6):1382–1386.
193. Lason, W., B. Przewlocka, and R. Przewlocki, The effect of gamma-hydroxybutyrate and anticonvulsants on opioid peptide content in the rat brain. Life Sci, 1983; 33 Suppl 1: 599–602.
194. Schmidt-Mutter, C., et al., Prodynorphin and proenkephalin mRNAs are increased in rat brain after acute and chronic administration of gamma-hydroxybutyrate. Neurosci Lett, 1999; 262(1):65–68.
195. Vayer, P., P. Mandel, and M. Maitre, Gamma-hydroxybutyrate, a possible neurotransmitter. Life Sci, 1987; 41(13):1547–1557.
196. Feigenbaum, J.J. and S.G. Howard, Naloxone reverses the inhibitory effect of gamma-hydroxybutyrate on central DA release in vivo in awake animals: a microdialysis study. Neurosci Lett, 1996; 218(1):5–8.
197. Crosby, G., et al., Naloxone pretreatment alters the local cerebral metabolic effect of gamma-hydroxybutyrate in rats. Brain Res, 1983; 275(1):194–197.
198. Devoto, P., et al., Naloxone antagonizes ethanol- but not gamma-hydroxybutyrate-induced sleep in mice. Eur J Pharmacol, 1994; 252(3):321–324.
199. Gobaille, S., et al., Characterization of methionine-enkephalin release in the rat striatum by in vivo dialysis: effects of gamma-hydroxybutyrate on cellular and extracellular methionine-enkephalin levels. Neuroscience, 1994; 60(3):637–648.
200. Dingledine, R., L.L. Iversen, and E. Breuker, Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. Eur J Pharmacol, 1978; 47(1):19–27.
201. Sethy, V.H., et al., Effect of anesthetic doses of gamma-hydroxybutyrate on the acetylcholine content of rat brain. Naunyn Schmiedebergs Arch Pharmacol, 1976; 295(1):9–14.
202. Oyama, T. and M. Takiguchi, Effects of gamma-hydroxybutyrate and surgery on plasma human growth hormone and insulin levels. Agressologie, 1970; 11(3):289–298.

203. Van Cauter, E. and G. Copinschi, Interrelationships between growth hormone and sleep. Growth Horm IGF Res, 2000; 10 Suppl B: S57–S62.
204. Van Cauter, E., et al., Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young Men. J Clin Invest, 1997; 100(3):745–753.
205. Gerra, G., et al., Flumazenil effects on growth hormone response to gamma-hydroxybutyric acid. Int Clin Psychopharmacol, 1994; 9(3):211–215.
206. Giustina, A. and J.D. Veldhuis, Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev, 1998; 19(6):717–797.
207. Volpi, R., et al., Muscarinic cholinergic mediation of the GH response to gamma-hydroxybutyric acid: neuroendocrine evidence in normal and parkinsonian subjects. Psychoneuroendocrinology, 2000; 25(2):179–185.
208. Brenneisen, R., et al., Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. J Anal Toxicol, 2004; 28(8):625–630.
209. Scharf, M.B., et al., Pharmacokinetics of gammahydroxybutyrate (GHB) in narcoleptic patients. Sleep, 1998; 21(5):507–514.
210. Borgen, L.A., et al., The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. J Clin Pharmacol, 2004; 44(3):253–257.
211. Couper, F.J. and B.K. Logan, Addicted to driving under the influence–a GHB/GBL case report. J Anal Toxicol, 2004; 28(6):512–515.
212. Borgen, L.A., et al., The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. J Clin Pharmacol, 2003; 43(1):59–65.
213. Xie, X.M., et al., $GABA_B$ receptor-mediated modulation of hypocretin/orexin neurons in mouse hypothalamus. J Physiol, 2006; 574(2):399–414.
214. Crunelli, V., et al., Cl- - and K + -dependent inhibitory postsynaptic potentials evoked by interneurones of the rat lateral geniculate nucleus. J Physiol, 1988; 399:153–176.
215. Mamelak, M., et al., A pilot study on the effects of sodium oxybate on sleep architecture and daytime alertness in narcolepsy. Sleep, 2004; 27(7):1327–1334.
216. Mamelak, M., J.M. Escriu, and O. Stokan, The effects of gamma-hydroxybutyrate on sleep. Biol Psychiatry, 1977; 12(2):273–288.
217. Scharf, M.B., et al., The effects and effectiveness of gamma-hydroxybutyrate in patients with narcolepsy. J Clin Psychiatry, 1985; 46(6):222–225.
218. Scrima, L., et al., Efficacy of gamma-hydroxybutyrate versus placebo in treating narcolepsy-cataplexy: double-blind subjective measures. Biol Psychiatry, 1989; 26(4):331–343.
219. Scrima, L., et al., The effects of gamma-hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. Sleep, 1990; 13(6):479–490.
220. de Lecea, L., et al., The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A, 1998; 95(1):322–327.
221. Sakurai, T., et al., Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell, 1998; 92(5):1 page following 696.
222. Wisor, J.P. and T.S. Kilduff, Molecular genetic advances in sleep research and their relevance to sleep medicine. Sleep, 2005; 28(3):357–367.
223. Lin, L., et al., The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell, 1999; 98(3):365–376.
224. Chemelli, R.M., et al., Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell, 1999; 98(4):437–451.
225. Thannickal, T.C., et al., Reduced number of hypocretin neurons in human narcolepsy. Neuron, 2000; 27(3):469–474.
226. Peyron, C., et al., A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med, 2000; 6(9):991–997.
227. Nishino, S., et al., Hypocretin (orexin) deficiency in human narcolepsy. Lancet, 2000; 355(9197):39–40.
228. Kilduff, T.S., Hypocretin/orexin: maintenance of wakefulness and a multiplicity of other roles. Sleep Med Rev, 2005; 9(4):227–230.
229. Saper, C.B., T.E. Scammell, and J. Lu, Hypothalamic regulation of sleep and circadian rhythms. Nature, 2005; 437(7063):1257–1263.
230. Finnimore, A.J., et al., The effects of the GABA agonist, baclofen, on sleep and breathing. Eur Respir J, 1995; 8(2):230–234.

231. LeWinter, R.D., et al., Fos activation by gamma-hydroxybuty in the rat CNS. Soc Neurosci Abstr 2005, p63.18.
232. Fuller, D.E. and C.S. Hornfeldt, From club drug to orphan drug: sodium oxybate (Xyrem) for the treatment of cataplexy. Pharmacotherapy, 2003; 23(9):1205–1209.
233. Wu, Y., et al., Gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acidB receptor (GABABR) binding sites are distinctive from one another: molecular evidence. Neuropharmacology, 2004; 47(8):1146–1156.
234. Wu, M.F., et al., Locus coeruleus neurons: cessation of activity during cataplexy. Neuroscience, 1999; 91(4):1389–1399.
235. John, J., et al., Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. Neuron, 2004; 42(4):619–634.
236. Siegel, J.M., et al., Neuronal activity in narcolepsy: identification of cataplexy-related cells in the medial medulla. Science, 1991; 252(5010):1315–1318.
237. Siegel, J.M., et al., Activity of medial mesopontine units during cataplexy and sleep-waking states in the narcoleptic dog. J Neurosci, 1992; 12(5):1640–1646.
238. Mileykovskiy, B.Y., L.I. Kiyashchenko, and J.M. Siegel, Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron, 2005; 46(5):787–798.
239. Mitler, M.M. and W.C. Dement, Cataplectic-like behavior in cats after micro-injections of carbachol in pontine reticular formation. Brain Res, 1974; 68(2):335–343.
240. Lai, Y.Y. and J.M. Siegel, Medullary regions mediating atonia. J Neurosci, 1988; 8(12):4790–4796.
241. Lai, Y.Y. and J.M. Siegel, Corticotropin-releasing factor mediated muscle atonia in pons and medulla. Brain Res, 1992; 575(1):63–68.

54
Sodium Oxybate for the Treatment of Narcolepsy

JED BLACK
Stanford University, Sleep Disorders Clinic, Stanford, California, U.S.A.

I. Overview

Sodium oxybate, most commonly known as gamma-hydroxybutyrate (GHB), has a three-decade history in the treatment of narcolepsy (1,2). Mamelak initially hypothesized that sodium oxybate could reduce sleep fragmentation in narcolepsy, through its known effects on increased sleep consolidation, slow-wave sleep augmentation, and rapid eye movement sleep facilitation (3). The seminal work of Mamelak and Broughton led to the beginnings of an understanding of sodium oxybate as a potent treatment, not only for improving sleep in patients with narcolepsy, but also for controlling cataplexy and enhancing daytime alertness. Further work by Mamelak, Scharf, Scrimma, Lammers and others provided long-term and controlled evidence supporting the efficacy of sodium oxybate for the treatment of narcolepsy (4–7). Recently, extensive research using large controlled and open-label studies has led to an appreciation of sodium oxybate as a primary treatment for the complex of narcolepsy symptoms (see reference list).

II. Introduction

Sodium oxybate is the sodium salt of gamma-hydroxybutyrate and is the preparation that has been used in narcolepsy clinical research and therapy. For the sake of simplicity, the name sodium oxybate will be used in this chapter including when referring to literature wherein authors have used another name.

Sodium oxybate is a four-carbon analog of gamma-aminobutyric acid (GABA) initially synthesized by Laborit who reported this work in 1960 (8). It was soon recognized to promote central nervous system (CNS) sedation, sleep consolidation, and deep (slow-wave) sleep, among other effects (9), and subsequently discovered to be an endogenous CNS substance (10,11). These properties led to the exploration of its utility in a variety of clinical settings. To date, it has been used effectively as a surgical anesthetic (12), and as treatment for ethanol withdrawal (13) or abstinence (14), fibromyalgia (15), and narcolepsy (2). Other less promising clinical exploration, often yielding equivocal or inconsistent results, has included the treatment of heroin dependence (16), schizophrenia (17) and depression (18). Additionally, intriguing preliminary work

has demonstrated that tissue-sparing effects are apparent when sodium oxybate is administered prior to, during, or even shortly following anoxia secondary to temporary circulatory arrest *in vivo* (19). This work may suggest future potential of this agent in the reduction of organ damage during ischemic events. Other areas of ongoing clinical research with sodium oxybate include chronic insomnia and Parkinson's disease.

This chapter reviews the clinical research on the use of sodium oxybate as treatment for narcolepsy from the initial work of Broughton and Mamelak through recent large industry-sponsored clinical trials.

III. Initial Exploration in Narcolepsy

The early work of Laborit and others characterizing the sleep-promoting effects of sodium oxybate led to speculation by Mamelak that this substance may improve sleep in patients with insomnia or fragmented sleep associated with a history of psychiatric illness. In 1973, Mamelak *et al*. described a placebo-controlled, crossover administration trial of low-dose sodium oxybate in 5 patients during nocturnal polysomnographic monitoring. In all subjects, an increase in delta sleep was observed and subjective improvement in sleep was reported. In addition, REM sleep latency was reduced, coupled with enhanced REM sleep consolidation (20).

Intrigued with these results, Mamelak and Broughton postulated that sodium oxybate may have similar beneficial effects on nocturnal sleep in narcolepsy and their initial exploration with in four subjects proved promising, not only in improved sleep continuity, but also in diminished cataplexy severity and frequency and in reduced daytime sleepiness (1).

When sodium oxybate treatment was extended to an additional 16 patients, a rapid improvement in subjective sleep quality and a gradual reduction in the frequency and severity of cataplexy were observed, as well as subjective improvements in daytime alertness (2). Continuous 48-hour ambulatory polysomnography (PSG) was performed in 14 of these patients at baseline and again 7 to 10 days after initiation of sodium oxybate treatment. PSG analysis revealed statistically significant changes in sleep including increased slow-wave sleep duration and sleep efficiency, and reductions in stage 1 sleep, sleep fragmentation, and REM latency and REM density compared to baseline. Additionally, daytime sleep duration, number of sleep episodes, slow-wave sleep and REM sleep were significantly reduced (3).

The sodium oxybate dose administered to these patients ranged from 3.75 to 6.25 g (approximately 50 mg/kg) per night and was titrated to optimal response. Due to its short half-life, sodium oxybate was administered in two nightly doses taken at bedtime and again during the night. In a few patients, a third dose was required to optimize sleep continuity throughout the night. Reported side effects included mild "hangover" effects during the first few days with subsequent resolution, and occasional urinary urgency with rare nocturnal enuresis.

IV. Further Open-Label Work

Scharf *et al*. expanded their initial work by enrolling 30 narcolepsy patients (17 women, 13 men) in an open-label sodium oxybate study designed to compare pretreatment

baseline measures to response following one and six months of treatment. This protocol required all patients to discontinue all medications used for the treatment of cataplexy following the initiation of sodium oxybate (n = 17), but stimulant medications were permitted (n = 24). The sodium oxybate dose was titrated to provide clinical improvement and ranged from 5 to 7 grams per night in 2 doses (some required a third dose to provide optimal sleep continuity) (4).

Following the first week of treatment, statistically significant improvements in cataplexy, hypnagogic hallucinations, sleep paralysis, and daytime sleep attacks were observed compared to baseline. After 12 weeks of treatment, cataplexy, hypnagogic hallucinations, and sleep paralysis were reduced by an average of approximately 90% and sleep attacks were reduced by 70% during this time. All symptoms were found to continue to improve until the end of the six-month observation period (4).

Analysis of PSG data after one month revealed significantly increased total sleep time, sleep efficiency, and percentage of slow-wave sleep compared to baseline. Significant reductions in total wake time, awakenings, and REM latency were also noted. In a subset of 12 patients who underwent repeat PSG after six months of treatment, changes in nocturnal sleep continued after the initial four-week treatment period.

In this study sodium oxybate was reported to be well-tolerated with side effects that included prolonged sleep paralysis in three patients following the first dose on the first night of treatment only, nocturnal enuresis in one patient and transiently increased libido in one patient (4).

V. Initial Long-Term Experience

Collaboration between Mamelak and Scharf during the mid-1980s resulted in a report describing the long-term, open-label experience of 48 patients with narcolepsy (21 men, 27 women) treated with 4.5 to 9 g of sodium oxybate nightly for durations of six months to nine years (5). Seventy-five percent of these patients became symptom-free over time using sodium oxybate as monotherapy in some patients, or with the addition of low-dose (<30 mg) of dextroamphetamine or methylphenidate in others. An additional 13 percent experienced an incomplete, but clinically meaningful response; only one of these required concomitant therapy (clomipramine 10 mg daily) for cataplexy. Six percent discontinued due to lack of adequate response and none due to side effects.

Long-term treatment was associated with few side effects. Sleep-walking occurred in six percent of patients and nocturnal enuresis in 4 percent during the first few nights of dosing.

VI. First Placebo-Controlled Trials

A. Scrima Trial (1989–1990)

In 1989 and 1990, Scrima et al. published the results of a blinded, placebo-controlled trial of low-dose sodium oxybate in narcolepsy (6,21). This study employed a 4-week (29-day) crossover design with a 6-day crossover (washout) period. Twenty patients (10 men and 10 women) were randomized to treatment with the relatively low-dose of 50 mg/kg sodium oxybate, or placebo, per night (25 mg/kg at bedtime and

25 mg/kg 3 hours later), during the first 4-week phase. Patients were then transferred to the alternate treatment for 4-weeks following the crossover period, in double-blind fashion. Prior to enrollment in the study, all patients reported cataplexy and excessive daytime sleepiness. Eleven were taking tricyclic antidepressants (TCA) for cataplexy; 18 patients were taking stimulant medication. All patients taking stimulant medications other than methylphenidate were switched to methylphenidate therapy before enrolling in the trial. Patients were maintained on <30 mg methylphenidate per day throughout the trial period.

A moderate placebo response was seen in cataplexy improvement, which may have been in part mediated by the continuance of a partial "rebound cataplexy" response following TCA discontinuation at baseline assessment; however, by week three of this trial, patients receiving sodium oxybate experienced a significant reduction in cataplexy compared to placebo (approximately 1 cataplexy event per day vs. 2 per day, respectively; $p = 0.022$). Improvements in cataplexy continued through the end of the trial. Abrupt discontinuation of sodium oxybate after 4-weeks of treatment did not precipitate rebound cataplexy during five days of follow-up monitoring.

In addition to a significant reduction in cataplexy events by the end of week 3, subjective "arousals from sleep" and hypnagogic hallucinations had decreased significantly compared to placebo ($p = 0.035$ and $p = 0.008$, respectively) by the end of week one of sodium oxybate treatment. This improvement continued for the duration of treatment. Objective measures of sleep were also significantly influenced by sodium oxybate administration. Specifically, these patients displayed decreased stage 1 ($p = 0.012$), increased stage 3 ($p = 0.008$), increased delta (stages 3 and 4 combined) sleep ($p = 0.049$), fewer stage shifts ($p = 0.002$), and fewer awakenings ($p = 0.006$) occurred following sodium oxybate versus placebo.

Despite substantial impact of low-dose sodium oxybate treatment on cataplexy, nocturnal sleep and ancillary narcolepsy symptoms, measures of daytime alertness (both subjective and objective) were not significantly improved compared to placebo in this study. These measures included the Stanford Sleepiness Scale (SSS), the number of sleep attacks per day, the number of naps per day, and the multiple sleep-latency test (MSLT); however, a trend toward reduced sleepiness on the MSLT ($p = 0.74$) as well as a significant increase in MSLT-wakefulness ($p = 0.03$) and a significant reduction in sleep-onset REM episodes ($p = 0.020$) during MSLT on the final day (day 29) of sodium oxybate versus placebo was seen.

Adverse events reported by patients receiving sodium oxybate therapy included nausea (15%), nausea with emesis (5%), nocturnal dizziness (5%), daytime dizziness (5%), weakness and fatigue (5%), urinary urgency (5%), and morning sluggishness and stiffness (5%). Interestingly, the total number of side effects reported by patients during the placebo period exceeded that reported during the sodium oxybate period. No serious adverse events were reported.

B. Lammers Trial (1990–1993)

Independent of any knowledge of the results of the trial by Scrima *et al*., Lammers *et al*. initiated a similar blinded, placebo-controlled, crossover study of low-dose sodium oxybate in patients with narcolepsy and cataplexy in the Netherlands (7). The trial design consisted of a 1-week baseline period followed by random assignment to 4

weeks of sodium oxybate or placebo, which was followed by a 3-week washout period. After the washout period, patients received the alternate treatment for 4 weeks. In this study, 24 patients (13 men and 11 women) were enrolled and administered 60 mg/kg sodium oxybate per night (30 mg at bedtime and 30 mg 4-hours later). Prior to inclusion, all patients reported excessive daytime sleepiness and cataplexy. Nine patients were taking stimulants and 7 were taking TCAs for cataplexy; 12 were on no concomitant medication during the study.

As in the Scrima study, a moderate placebo response was observed in cataplexy improvement; however, by week 3, greater improvement was demonstrated in patients receiving sodium oxybate treatment compared to those patients receiving placebo treatment. These improvements were maintained throughout the trial and the magnitude of improvement was similar to that reported in the Scrima trial.

In general, subjective and objective nocturnal sleep-related (PSG) measures also demonstrated a response consistent with previous controlled and uncontrolled studies. A marked reduction in hypnagogic hallucinations ($p = 0.008$), an increase in slow-wave sleep (stages 3 and 4; $p = 0.053$), a reduction in REM-sleep awakenings ($p = 0.016$), and a decrease in time spent awake during REM ($p = 0.007$) was observed.

Of particular interest in this study, all measures of subjective daytime sleepiness were substantially improved despite the low doses of sodium oxybate used. Patient-reported number of sleep attacks ($p = 0.001$) and severity of daytime sleepiness ($p = 0.028$) were reduced; however, MSLT results were not significantly different from placebo, although only 7 subjects had adequate MSLT data for analysis.

Adverse effects related to sodium oxybate treatment in this study were apparently rare. The adverse events reported by the authors were an episode of a prolonged hypnagogic hallucination with sleep paralysis in one patient during the first week of treatment and "loss of weight" in one patient during the first two weeks of treatment.

VII. Formal Development of Sodium Oxybate for the Treatment of Narcolepsy

Owing largely to the ongoing effort of, and information supplied by Dr. Martin B. Scharf in the United States of America, coupled with the clinical exploration and pilot clinical trial reports summarized above, the US Food and Drug Administration (FDA) solicited the participation of Orphan Medical, Inc., to provide the necessary multi-center clinical trials required for regulatory approval of sodium oxybate for the treatment of narcolepsy. At that time, no medication had undergone the rigorous evaluation required for approval in cataplexy treatment. In response, Orphan Medical initially evaluated the efficacy of sodium oxybate for the treatment of cataplexy, and subsequently for the treatment of excessive daytime sleepiness and disrupted nocturnal sleep in patients with narcolepsy.

This section summarizes the results of several large, multi-center sodium oxybate clinical trials conducted by Orphan Medical in approximately 1000 patients with narcolepsy during the past decade. The results of these trials provide definitive data on the efficacy of sodium oxybate for the treatment of excessive daytime sleepiness, cataplexy, and nocturnal sleep fragmentation, the three dominant symptoms of narcolepsy, as well as on its tolerability and safety profile. For the sake of clarity and simplicity, the

results of the six most-relevant multi-center clinical trials will be summarized under the symptom subcategories of Cataplexy, Excessive Daytime Sleepiness and Disturbed Nocturnal Sleep. Some of these trials evaluated more than one symptom and will therefore be reviewed in more than one subsection.

A. Cataplexy

Short-Term Administration Studies

In the first double-blind placebo-controlled multi-center trial of sodium oxybate for the treatment of narcolepsy the primary outcome measure was the number of cataplexy events per week, and secondary measures included Epworth Sleepiness Scale (ESS)(22), number of inadvertent naps/sleep attacks, and number of awakenings during nocturnal sleep (23). Following a 14 to 21-day baseline period, 136 patients (57 men and 79 women) with narcolepsy and cataplexy were randomly assigned to receive placebo or a total of 3, 6, or 9 g of sodium oxybate per night, taken in equally-divided doses at bedtime and 2.5 to 4 hours later, for a four-week period. Patients receiving prior treatment with anticataplectic medications underwent gradual withdrawal of these medications followed by a period of cataplexy stabilization before undergoing baseline evaluation. Patients receiving prior stimulant medication(s) (113 subjects or 83.1 %) continued at a steady dose throughout the trial. At the completion of the 4-week double-blind treatment phase, patients abruptly discontinued this treatment for a 3 to 5-day period of observation.

As was observed in previous controlled trials, sodium oxybate significantly reduced the number of cataplexy episodes per week compared to placebo. Moreover, a clear dose-response relationship was observed with the greater reduction in cataplexy events occurring at the 9 g dose ($p = 0.0008$). A detailed analysis of data generated with this study suggested that greater improvement in cataplexy occurred during weeks 3 and 4 of treatment than occurred during weeks 1 and 2. Additionally, subsequent open-label experience (summarized later) suggested that maximal improvement at any given dose may not occur by week 4 of sodium oxybate treatment, but may require 8 to 12 weeks in some patients. In contrast to clinical experience with traditional agents used to treat cataplexy, a complete lack of "rebound cataplexy" following abrupt discontinuation of sodium oxybate after 4 weeks of continuous administration was observed.

The finding that a 4-week treatment duration was insufficient to assess full response to a particular dose of sodium oxybate was rigorously evaluated in a second, larger multi-center study of similar design, wherein treatment duration was lengthened to 8 weeks (24). In that study, 228 patients (79 men and 149 women) received placebo or sodium oxybate in two equally-divided doses at bedtime and 2.5–4 hours later. To increase the tolerability of the medication, all patients who were randomly assigned to receive active medication began at 4.5 g and increased by 1.5 g per week until the assigned dose of 4.5, 6, or 9 g nightly was obtained. Similarly, those assigned to placebo were randomized to 4.5 g, 6 g, or 9 g mock sodium oxybate (placebo) and titrated by 1.5 g per week until assigned placebo dose was achieved (Fig. 1).

The results of this 8-week trial again demonstrated a clear dose-response effect on cataplexy reduction, but provided additional evidence for a "time-on-drug" effect, which is evident for up to 8 weeks of sodium oxybate treatment (Fig. 2). Moreover,

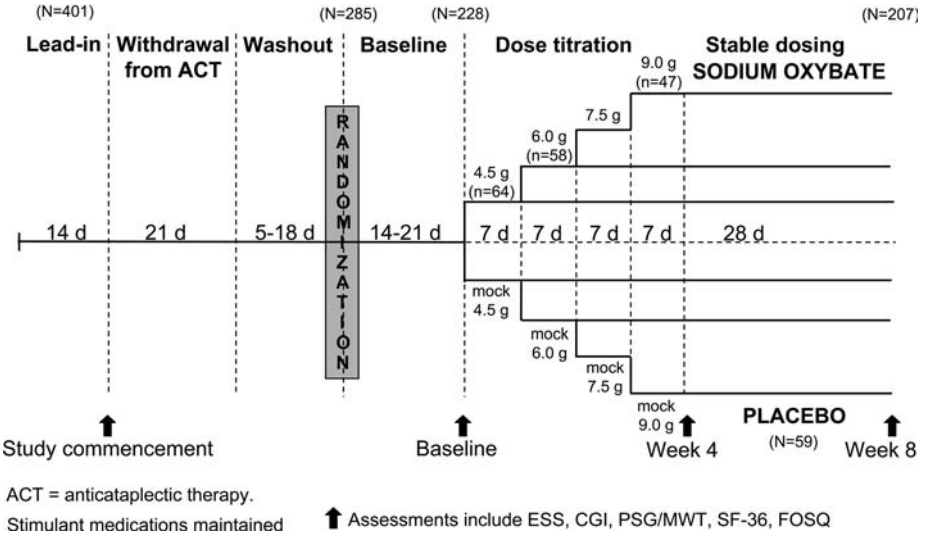


Figure 1 8-Week controlled trial design for sodium oxybate. (See text.)

by eight weeks of treatment, the 4.5 g dose provides statistically significant reduction ($p = 0.001$) in cataplexy, in addition to the 6 g ($p = 0.001$) and 9 g ($p = 0.001$) doses. By the 8-week assessment, the median reduction in number of cataplexy events per week was 85% in patients randomized to 9 g. Measures of daytime sleepiness and nocturnal sleep were also assessed in this study and are reviewed under those respective sections.

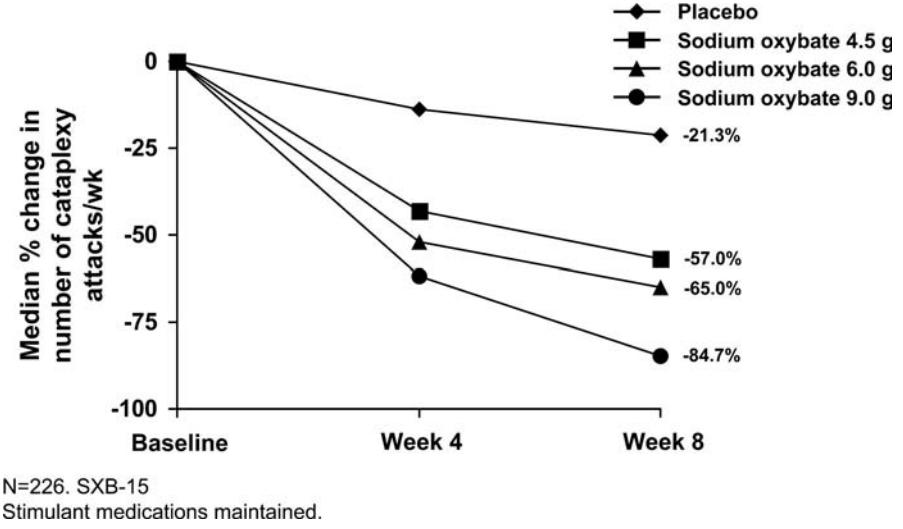


Figure 2 Dose-response effect and time effect of sodium oxybate on cataplexy (8-week trial).

Chronic Administration Studies

Two large multi-center trials evaluated the long-term benefit of sodium oxybate for the treatment of cataplexy for six months or more. The first study enrolled 118 of the patients who had participated in the 4-week multi-center trial (described above) in an open-label (unblinded) fashion for 12 months (25). All patients began medication at 6 g per night, in two equally-divided doses, and sodium oxybate dose was held constant or adjusted up (7.5 to 9 g) or down (4.5 to 3 g) as deemed appropriate by the research physician to optimize treatment effect and minimize side effects. Long-term reductions in cataplexy were of substantially greater magnitude than those observed during the 4-week trial. By the end of 12 months, a greater than 90% reduction in the median number of cataplexy attacks per week, for the entire group of participants, was observed.

The second chronic administration multi-center trial enrolled 55 patients with narcolepsy/cataplexy who had received continuous treatment with sodium oxybate for 7 - 44 months duration; mean duration was 21 months (26). Patients continued to receive their stable dose of sodium oxybate in single-blind fashion during a 2-week baseline phase. Subsequently, patients were randomized to continue active sodium oxybate at their respective dose or to receive an equivalent volume of placebo sodium oxybate solution during a 2-week period in a double-blind fashion.

In this trial, patients randomized to placebo experienced a significant increase in cataplexy events ($p < 0.001$) compared to those randomized to continued active sodium oxybate treatment. These results indicate that sodium oxybate continues to provide effect treatment for many months to many years without evidence of tachyphylaxis.

Of interest is the observation that patients randomized to placebo and abruptly discontinued from sodium oxybate did not experience rebound cataplexy, despite prior long-term treatment. Conversely, an apparent carryover treatment effect continued such that, by the end of 2 weeks, these patients, while significantly worse than during treatment, still had not deteriorated to pretreatment severity of cataplexy. This finding indicates that the exacerbation in cataplexy occurring in the placebo group was not a result of a "rebound" effect commonly seen with other agents used for cataplexy treatment, and demonstrates that sodium oxybate is effective long-term therapy.

In summary, the results of these four large multi-center trials demonstrate the substantial efficacy of sodium oxybate in the treatment of cataplexy. The extent of symptom improvement, coupled with the percentage of patients improved and the sustained effect observed over long-term evaluation, exceeds that reported in published trials of any other anticataplectic agent.

B. Excessive Daytime Sleepiness

Short-Term Administration

In addition to assessing the effect of sodium oxybate on cataplexy provided by the 4-week controlled multi-center trial described in the previous cataplexy section, the impact on subjective measures of daytime sleepiness was also assessed in that study (23). A clear dose-response improvement occurred in all measures assessed, including the Epworth Sleepiness Scale (ESS) and the number of inadvertent naps/sleep attacks,

reaching statistical significance at the 9 g dose for both measures ($p = 000.1$; $p = 0.0122$, respectively).

To further explore the dose-relationship of sodium oxybate-induced improvement in sleepiness and to evaluate its benefit using an objective measure of sleepiness, a subsequent multi-center study subjected patients to a "forced" dose titration of sodium oxybate (27) in which 25 patients (7 men and 18 women) were evaluated. Following a baseline evaluation, each subject was administered 4.5 g sodium oxybate nightly in two equally-divided doses for 4 weeks. This was followed by 6 g nightly for 2 weeks, then 7.5 g nightly for 2 weeks, and finally 9.0 g for 2 weeks. Measures assessed included ESS and nocturnal PSG, both performed before and after the baseline evaluation period, after the initial 4.5 g dose and on the last night of 4.5, 6, 7.5, and 9 g doses. The maintenance of wakefulness test (MWT) was also performed during the same visits as PSG with the exception of the beginning of the 4.5 g dose as well as the last nights of the 6 and 7.5 g doses.

Significant improvement in ESS group means occurred with all doses of sodium oxybate used ($p < 0.001$) compared to baseline and with the 7.5 g ($p < 0.001$) and 9 g ($p < 0.001$) doses when compared to prebaseline assessment. The mean results of the 20-minutes. MWT also evidenced robust improvement in sleepiness at doses assessed compared to baseline (4.5 g, $p < 0.05$; 9 g, $p < 0.001$). The mean MWT sleep latency in the 9 g dose group more than doubled from 4.5 min at baseline to 10.6 min.

A third multi-center trial, described in the previous cataplexy section, assessed the impact of nightly doses 4.5, 6, and 9 g sodium oxybate on daytime sleepiness during an 8-week double-blind study (Fig. 1) (24). ESS (Fig. 3) and number of inadvertent naps/sleep attacks were significantly improved at both 6 and 9 grams (for each,

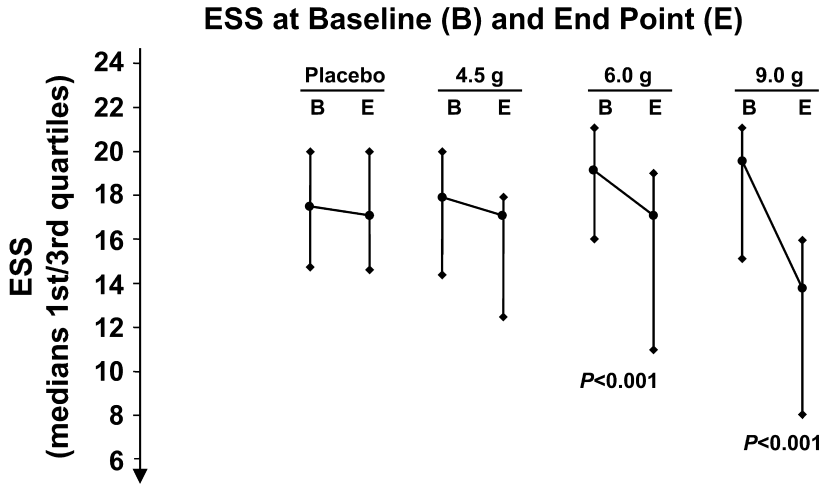
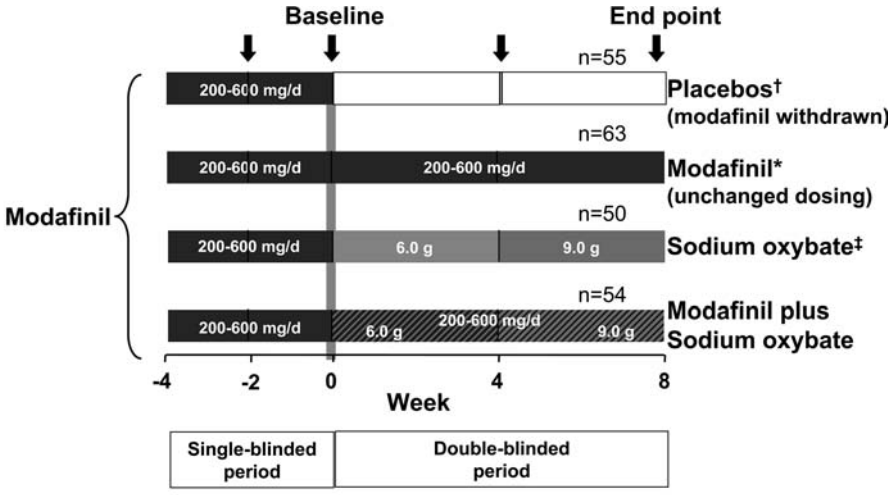


Figure 3 Dose-response effect of sodium oxybate on Epworth Sleepiness Scale (ESS) (8-week trial).



N=222. SXB-22.
*Placebo: sodium oxybate; †Placebos: modafinil + sodium oxybate; ‡Placebo: modafinil.

Figure 4 8-Week sodium oxybate vs. modafinil trial design.

$p < 0.001$). Additionally, the 40-minute MWT increased from 7.6 minutes at baseline to 17.7 minutes at nightly doses of 9 g ($p < 0.001$), demonstrating substantial improvement in daytime sleepiness.

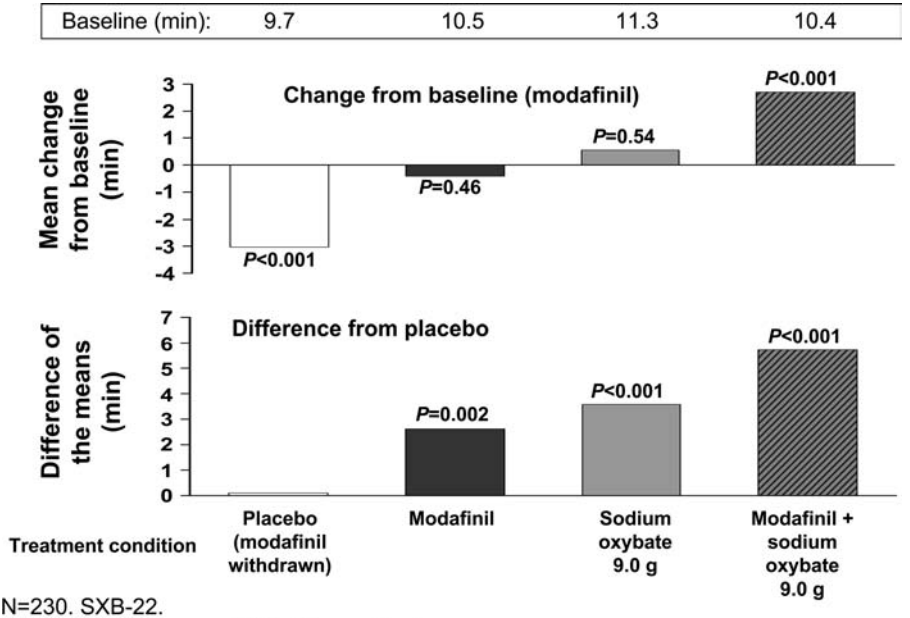
A fourth multi-center evaluation of sodium oxybate effect on daytime sleepiness has been recently completed and is not described in the preceding sections (Orphan Medical, data on file). This double-blind placebo-controlled, double-dummy trial assessed the efficacy of sodium oxybate in patients previously using modafinil (Fig. 4). In that trial, 228 narcolepsy patients (107 men and 115 women), with or without cataplexy, who had been on stable, effective and well-tolerated doses of modafinil for daytime sleepiness, were randomized to one of 4 treatment groups for eight weeks: modafinil (with placebo sodium oxybate), sodium oxybate (with placebo modafinil), sodium oxybate and modafinil, or placebo. Patients were not receiving stimulant treatment with any agent other than modafinil and were required to be taking a daily dose of modafinil for three weeks or more prior to study participation. Patients randomized to the modafinil arm continued their previous dose of modafinil (200–600 mg daily) along with placebo sodium oxybate for the 8-week study period. Patients randomized to sodium oxybate received 6 g nightly in two equally-divided doses at bedtime and 2.5–4 hours later for 4 weeks followed by 9 g nightly for the remaining 4 weeks along with placebo modafinil. Those randomized to combination treatment received modafinil at study entry dose combined with sodium oxybate 6 g nightly for the first 4 weeks and then 9 g nightly the remaining 4 weeks. The patients randomized to placebo received both placebo modafinil and placebo sodium oxybate for the entire 8-week period.

As expected, the change from modafinil to placebo resulted in a significant decrease in mean average MWT sleep latency from 9.74 min at baseline to 6.87 min after 8 weeks ($p < 0.001$). As might be expected, the patients randomized to continued modafinil alone demonstrated no change in sleep latency. In the group of patients treated with sodium oxybate alone, there was a nonsignificant trend toward an increase in sleep latency, demonstrating that it was as efficacious as the previously administered modafinil. In contrast, the patients randomized to receive the sodium oxybate/modafinil combination evidenced an increase from 10.43 min to 12.91 min ($p < 0.001$), suggesting an additive beneficial effect (Fig. 5). The sodium oxybate group also demonstrated a significant decrease in number of inadvertent daytime nap/sleep episodes and a decrease in median average ESS scores from 15 to 12.0 while the sodium oxybate/modafinil group decreased from 15.0 to 11.0 (for both, $p < 0.001$) (Fig. 6).

Additional measures that are not reviewed in this section, but that were performed in these multi-center trials and that were significantly improved in a dose-response fashion include the investigator-rated global assessment of clinical change and the patient-rated ability to concentrate and overall condition.

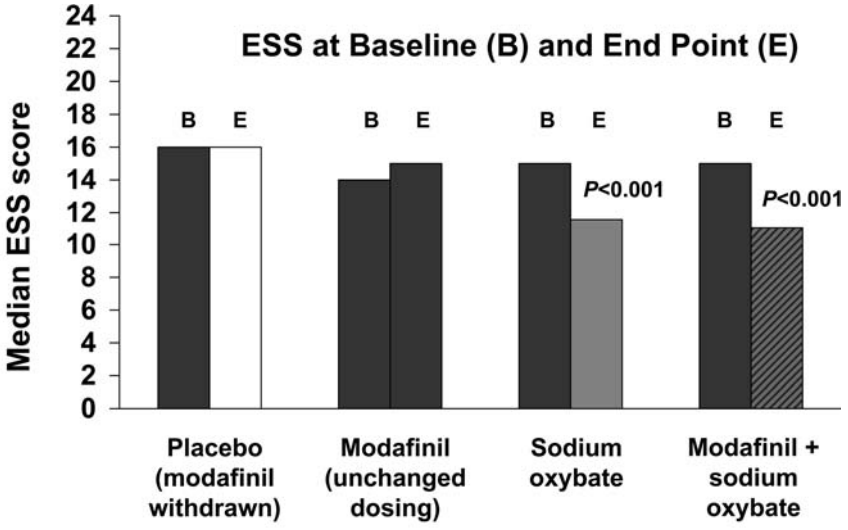
Chronic Administration

The 12-month open-label study described in the previous cataplexy section included bi-monthly ESS measures (25). In that study, the mean ESS for patients prior to trial entry



N=230. SXB-22.
MWT = Maintenance of Wakefulness Test.

Figure 5 Sodium oxybate versus modafinil: 40-minute MWT sleep latency.



N=222. SXB-22.
EDS = excessive daytime sleepiness; ESS = Epworth Sleepiness Scale.

Figure 6 8-Week sodium oxybate versus modafinil: ESS.

was 18 and was significantly improved to 13 ($p < 0.001$) during the 12-month period, with little fluctuation over time and no evidence of tachyphylaxis.

To summarize, several large multi-center clinical trials evaluating the impact of sodium oxybate on multiple measures of daytime sleepiness demonstrated consistent short-term and long-term improvement when administered in combination with stimulant therapy or when used as the only wake-promoting treatment. In addition, compared to modafinil, sodium oxybate as monotherapy appears to produce equal or greater improvement in daytime sleepiness in patients with narcolepsy with, or without co-morbid cataplexy.

C. Disturbed Nocturnal Sleep

Short-Term Administration

The three multi-center trials described in the previous sections characterize the effects of sodium oxybate on nocturnal sleep. The first of these was the open-label "forced" dose-titration pilot study wherein 25 patients with narcolepsy were subjected to nocturnal PSG evaluation following the first dosing of 4.5 g sodium oxybate, after four weeks of continuous treatment with 4.5 g, then during the final nights after each of two weeks of treatment with 6, 7.5, and then 9 g nightly (27). Results of this study parallel those published by Mamelak almost 30 years ago (1).

The main findings of this trial included a robust, dose-related and statistically significant increase in slow-wave sleep duration at 7.5 and 9 g (for each, $p < 0.05$), and in delta power (a measure of the rate of occurrence of approximately 0.5 to 4 Hz EEG

activity coupled with the amplitude of the waves in this frequency range) across all doses. Additionally, REM-sleep latency was significantly decreased and REM-sleep duration significantly increased during the first night of 4.5 g sodium oxybate administration (for each, $p < 0.05$). In contrast, with longer administration the shortened REM-latency abated and total REM-sleep duration decreased in a dose-dependent manner such that at 9 g the REM-sleep duration was moderately, but statistically significantly reduced relative to the 4.5 and 6 g doses ($p < 0.005$). No consistent effects were seen on sleep-latency, total sleep duration, stage 1 or 2 duration, or number of awakenings, across all doses, in this study.

The next multi-center evaluation of sodium oxybate on nocturnal sleep was conducted in conjunction with the previously described 8-week parallel-group study comparing 4.5, 6, and 9 g sodium oxybate treatment with placebo in 226 patients (24) (Fig. 7). As expected from results of prior studies, slow-wave sleep (4.5 g, $p < .05$; 6 and 9 g, $p < 0.001$) and delta power (4.5 g, $p = 0.006$; 6 and 9 g: $p < 0.001$) were significantly increased at all doses of sodium oxybate compared to placebo. REM-sleep duration was again moderately decreased ($p < 0.05$) at the 9 g dose only. With this larger study population a significant decrease in number of awakenings ($p < 0.05$) and stage 1 sleep ($p < 0.001$) occurred at the 6 and 9 g doses and a modest increase in total sleep duration ($p < 0.05$) was observed at the 9 g dose (Orphan Medical, data on file).

Of note are the observations that a modest correlation between the nocturnal increase in slow-wave sleep and the reduction in ESS score was the only significant correlation found between sodium oxybate-induced sleep changes and improvements in sleepiness in the first trial, and that no correlation was found between measures of sleep and improvements in sleepiness at any dose in the much larger second trial

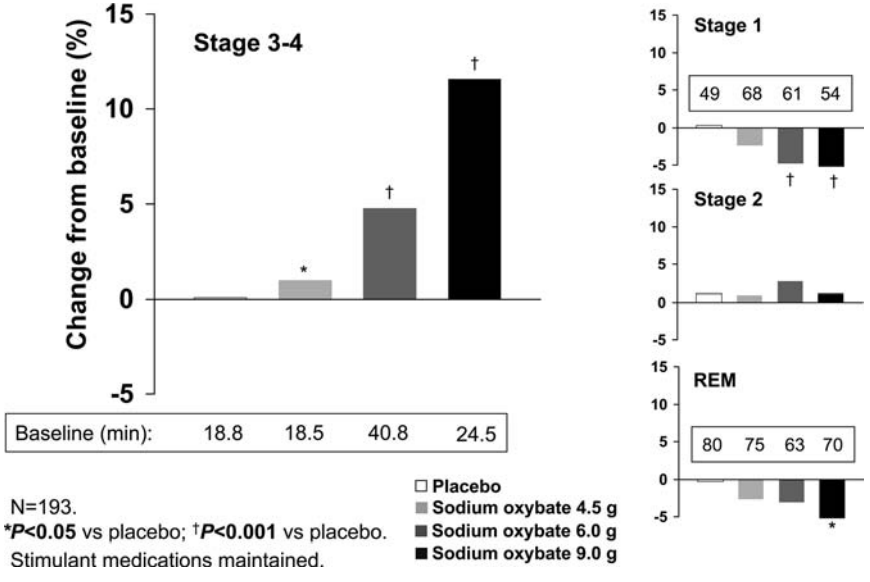


Figure 7 Sodium oxybate impact on sleep stages.

(Orphan Medical, data on file). This finding leads one to speculate that the impact of sodium oxybate on excessive daytime sleepiness may be independent of its effect on nocturnal sleep or that our methods of sleep analysis are inadequate.

The third trial, in which sodium oxybate was compared to placebo, modafinil, and a combination of sodium oxybate and modafinil, produced changes in nocturnal sleep that were consistent with previous trial results (Orphan Medical, data on file). Moreover, these effects were seen with sodium oxybate independent of modafinil co-administration.

In summary, results of multi-center trials corroborate earlier work and demonstrate a consistent effect of sodium oxybate on slow-wave sleep activity yielding a substantial, dose-related increase in slow-wave sleep duration and delta power. Additionally, a dose-related reduction in stage 1 sleep and number of awakenings is apparent in the larger study as well as a modest increase in total sleep duration and reduction in REM-sleep duration at the 9 g dose. Meaningful studies of the impact of sodium oxybate on sleep for periods exceeding 8 weeks have not been conducted; however, clinical experience suggests that changes in sleep documented in short-term studies persist during chronic administration.

D. Hypnagogic/Hypnopompic Hallucinations and Sleep Paralysis

Clinical experience suggests that acute administration of sodium oxybate may precipitate prolonged hypnagogic hallucinations or sleep paralysis in some patients. In such cases, chronic administration generally results in an improvement, compared to pretreatment, of both symptoms. Additionally, Lammers reported a significant reduction in these symptoms with sodium oxybate treatment (7). Despite these findings, no significant improvement in these symptoms has been found in any of the larger, multi-center trials. This lack of improvement may stem from the fact that the frequency of these symptoms was quite low. In one study, the median number of weekly hypnagogic hallucinations ranged from 1.13 to 3.05 across all dose groups while the median number of sleep paralysis episodes ranged from 0.5 to 2.0 (24) and a so-called "floor" effect may have limited further improvement.

VIII. Safety and Tolerability in Narcolepsy

Data from the two eight-week large multi-center placebo-controlled trials, described in preceding sections, provide the most rigorous evaluations of the safety and tolerability of sodium oxybate treatment in narcolepsy.

In the first of these two, of 228 patients enrolled to receive placebo or sodium oxybate, adverse events with greater than 5% incidence included nausea, dizziness and enuresis which appeared to be dose-related (24). Events occurring with less than 5% incidence included feeling drunk (2 events), contusion (3 events), back pain (5 events), muscle cramp (4 events), somnolence (8 events), disturbance in attention (6 events), dysarthria (2 events), tremor (2 events), disorientation (5 events), sleep walking (3 events), dyspnea (5 events) and snoring (4 events). Twenty-one patients discontinued the trial due to an adverse event in the placebo (1 patient), 4.5 gm (1 patient), 6 gm (4 patients) and 9 gm (15 patients) dose groups and only 14 unique

events occurred with a frequency significantly greater than placebo; however, some patients assigned to the 6 and 9 g dose groups discontinued during the titration phase and did not actually reach their assigned dose.

Six serious adverse events (SAEs) were reported during the study. Three occurred during the single-blind placebo phase of the trial. Three other SAEs occurred during the double-blind phase of the study. One involved a 33-year-old female who experienced acute onset of fever, chills, and shortness of breath associated with pneumonitis while at the 6 g dose during the double-blind phase of the trial. She discontinued study drug for 2 days while hospitalized and treated with IV antibiotics and then resumed her study medication. Another SAE involved a 57-year-old female with many years of vertigo prior to randomization to 9 g sodium oxybate who fractured her ankle in a fall while walking to the bathroom approximately 3 hours after going to bed. The third SAE occurred in a 60-year-old female assigned to the 4.5 g dose whose serum alanine amino transferase (ALT) and aspartate amino transferase (AST) concentrations were elevated at the conclusion of the trial, compared to baseline. These gradually normalized following completion of the trial (24).

In the second eight-week trial, patients taking modafinil chronically with good tolerability were randomized to 4 treatment arms for comparison: placebo, sodium oxybate, modafinil, or combination sodium oxybate with modafinil. At least one adverse event was reported in 151 of 231 treated patients (65.4%) who entered the double-blind treatment phase of the study. Compared to the incidence of adverse events reported in the sodium oxybate (60%), modafinil (54.0%) or placebo groups (69.6%), a somewhat greater number of adverse events were reported in the combination sodium oxybate/modafinil group (78.9%). Among all patients, the most common treatment-emergent adverse events included headache (15.2%), nausea (11.7%), dizziness (9.1%), nasopharyngitis (6.1%), vomiting (6.1%), and somnolence (5.6%). Of these, only nausea, vomiting and dizziness were statistically significant different between treatment groups. Nausea and vomiting occurred with highest frequency in the sodium oxybate groups while the incidence of dizziness was highest in the sodium oxybate/modafinil group (Orphan Medical, data on file).

The number of patients who withdrew from this study early as the result of a treatment-emergent adverse event or serious adverse event was greatest in the sodium oxybate/modafinil group (6) compared to the sodium oxybate (4), modafinil (2), or placebo groups (1). Adverse events reported in 4 patients included (MedDRA coding terminology): pregnancy (protocol violation), abdominal pain, palpitations, and a psychotic disorder due to a general medical condition (narcissistic personality disorder); however, the only event deemed drug-related was the psychotic disorder, which occurred in the sodium oxybate/modafinil group.

IX. Pharmacokinetics, Drug Interactions and Dosing Considerations

Careful evaluation of acute and chronic (eight weeks, nightly) dosing of 4.5 g of sodium oxybate in patients with narcolepsy reveals a very similar time to maximal plasma concentration (Tmax) of 0.5 to 0.75 hours and no change in the half-life of

0.67 hours (29). Acute and chronic dosing maximal plasma concentrations (Cmax) and plasma areas under the curve (AUC) are also similar. A nonlinear dose-response pharmacokinetic (PK) profile is observed with multiple nightly doses, and as doses are increased from 4.5 to 9 g (30). A high-fat meal immediately before dosing reduces absorption and plasma AUC by as much as one-third. No gender differences in PK profile have been observed (31). Pharmacokinetic studies in children have not been performed.

Animal studies indicate that metabolism is the major elimination pathway for sodium oxybate, producing carbon dioxide and water via the tricarboxylic acid (Krebs) cycle and secondarily by beta-oxidation. The clearance of sodium oxybate is almost entirely by biotransformation to carbon dioxide, which is then eliminated by expiration (32). On average, less than 5% of unchanged drug appears in human urine within 6 to 8 hours after dosing. Fecal excretion is negligible. Because the kidney does not play a significant role in the excretion of sodium oxybate, no effect of renal function on sodium oxybate pharmacokinetics would be expected; however, no pharmacokinetic studies in patients with renal dysfunction have been conducted.

Sodium oxybate undergoes significant presystemic (hepatic first-pass) metabolism. AUC and elimination half-life of sodium oxybate have been found to be significantly greater in cirrhotic patients, with or without ascites, compared to control subjects (AUC approximately double for both types of cirrhosis versus control subjects; meant $1/2 = 59$ and 32 versus 22 minutes, respectively) (33). Based on these data, it is prudent to reduce the starting dose of sodium oxybate by one-half in patients with liver dysfunction.

Drug interaction studies in healthy adults have demonstrated no pharmacokinetic interactions between sodium oxybate and protriptyline hydrochloride, zolpidem tartrate (34), or modafinil (35). However, pharmacodynamic interactions with these drugs have not been excluded. The only absolute contraindication for sodium oxybate administration is in succinic semialdehyde dehydrogenase deficiency. This is very rare congenital inborn error of metabolism that precipitates severe mental and developmental retardation during early childhood accompanied by ataxia and hypotonia (36).

Clinical experience combined with the results of clinical trials reviewed in this chapter demonstrate that tolerability is optimized when sodium oxybate is initiated at lower doses of 4.5 to 6 g nightly in equally-divided doses at bedtime and 2 to 4 hours later and gradually increased as needed. Results from clinical trials demonstrate that optimal cataplexy response is not achieved until a patient has been maintained on a given dose for 4 to 12 weeks. Therefore, if cataplexy is the target symptom, dosing should be held constant for 1 to 3 months, once approximately 6 g of nightly dosing is achieved, before further increasing the dose. In contrast, optimal effects on nocturnal sleep and daytime alertness may occur within 4 to 8 weeks of dosing; therefore, for target symptoms of sleepiness or nocturnal sleep fragmentation, more rapid titration, as needed, can be implemented. On occasion, doses higher than 9 g will be required to achieve optimal improvement in narcolepsy symptoms. In such cases, titration should proceed with careful patient monitoring. Of critical note, however, is the need to continue increasing the dose every 4 to 12 weeks until clinic benefit is optimized or unresponsiveness demonstrated. Slower dose titration has been recommended by some researchers (6).

X. Illicit Use and Abuse Potential

Many case reports and limited case series have reported the illicit use of gamma-hydroxybutyrate including: at social gatherings (e.g., "rave" parties) to promote dis-inhibition, in conjunction with stimulants (including MDMA—"ecstasy") to diminish the severity of the stimulant-induced "crash," to promote sedation to facilitate sexual assault (37). Heavy, long-term use with frequent around-the-clock consumption has resulted in addiction and/or withdrawal (38). Carefully performed surveys demonstrate the use of gamma-hydroxybutyrate in date-rape is approximately one-tenth that of alcohol and one-half that of benzodiazepines (39), and its potential for other abuse and addiction is far less than that of alcohol or benzodiazepines (40,41). Additionally, true addiction appears to be rare and to occur usually in polysubstance abusers and to require repeated dosing throughout the 24-hour period (42).

Since the commercial availability of sodium oxybate in the United States, illegal diversion to another individual for illicit use, or addiction in a patient with narcolepsy has not been reported. Withdrawal symptoms do not occur following abrupt discontinuation of sodium oxybate in narcolepsy treatment, even after many weeks or months of nightly administration (26,43). When used as treatment for narcolepsy, sodium oxybate has limited, if any, abuse or illicit use potential.

XI. Conclusion

The vast published clinical research dataset coupled with substantial clinical experience over 30 years demonstrates that sodium oxybate constitutes a very important and effective treatment of narcolepsy. When appropriately dosed for an adequate duration, sodium oxybate robustly attenuates or eliminates cataplexy, reduces daytime sleepiness, and improves nocturnal sleep. Our current use of this agent is as first-line therapy for excessive daytime sleepiness and/or cataplexy in all patients with narcolepsy of any age, whether the patient is treatment-naive or has received previous inadequate treatment. Additionally, despite our initial concerns with the safety of sodium oxybate, due largely to reports ultimately deemed inaccurate, this substance has proven relatively safe in the acute and chronic management of narcolepsy symptoms.

References

1. Update – Bogan RK. Sodium oxybate, alone and in combination with modafinil, produces significant changes in sleep architecture in narcolepsy. SLEEP 2005; 28 (Suppl): A242.
2. Update – Black J. Nightly administration of sodium oxybate is effective for the treatment of excessive daytime sleepiness in narcolepsy. SLEEP 2005; 28 (Suppl): A218.
3. Broughton R and Mamelak M. Gamma-hydroxybutyrate in the treatment of compound narcolepsy: a preliminary report. In Guilleminault C, Dement WC, Passouant P, Eds. Narcolepsy. Spectrum, New York 1976:659–667.
4. Broughton R and Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. Can J Neurol Sci 1979; 6:1–6.
5. Broughton R and Mamelak M. Effects of nocturnal gamma-hydroxybutyrate on sleep/waking patterns in narcolepsy-cataplexy. Can J Neurol Sci 1980; 7:23–31.

6. Scharf MB, Brown D, Woods M, Brown L, Hirschowitz J. The effects and effectiveness of gamma-hydroxybutyrate in patients with narcolepsy. J Clin Psychiatry 1985; 46:222–225.
7. Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. SLEEP 1986; 9:285–289.
8. Scrima L, Hartman PG, Johnson FH Jr, Hiller FC. Efficacy of gamma-hydroxybutyrate versus placebo in treating narcolepsy-cataplexy: double-blind subjective measures. Biol Phychiatry 1989; 26:331–343.
9. Lammers GJ, Arends J, Declerck AC, Ferrari MD, Schouwink G, Troost J. Gammahydroxybutyrate and narcolepsy: a double-blind placebo-controlled study. SLEEP 1993; 16:216–220.
10. Laborit H, Jouany JM, Gerard J, Fabiani F. Generalities concerning the experimental study and clinical use of gamma hydroxybutyrate of Na. Agressologie 1960; 1:397–406.
11. Laborit H. Correlations between protein and serotonin synthesis during various activities of the central nervous system (slow and desynchronized sleep, learning and memory, sexual activity, morphine tolerance, aggressiveness, and pharmacological action of sodium gamma-hydroxybutyrate). Res Commun Chem Pathol Pharmacol 1972; 3:51–81.
12. Bessman SH, Fishbein WN. Gamma-hydroxybutyrate, A normal brain metabolite. Nature 1963; 200:1207–1208.
13. Roth RH, Formation and regional distribution of gamma-hydroxybutyric acid in mammalian brain. Biochem Pharmacol 1970; 19:3013–3019.
14. Kleinschmidt S, Grundmann U, Knocke T, Janneck U, Kreienmeyer J, Kulosa R, Larsen R. Total intravenous anaesthesia using propofol, gamm-hydroxyburtyrate (GHB) and sufentanil in patients undergoing coronary artery bypass graft surgery: a comparison in patients with unimpaired and impaired left ventricular function. Eur J Anesthesiol 1998; 15:559–564.
15. Nimmerrichter AA, Walter H, Gutierrez-Lobos KE, Lesch OM. Double-blind controlled trial of gamma-hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. Alcohol Alcohol 2002; 37:67–73.
16. Addolorato G, Castelli E, Stefanini GF, Casella G, Caput F, Marsigli L, Bernardi M, Gasbarrini G. An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. Alcohol Alcohol 1996; 31:341–345.
17. Scharf MB, Baumann M, Berkowitz DV. The effects of sodium oxybate on clinical symptoms and sleep patterns in patients with fibromyalgia. J Rheumatol 2003; 30:1070–1074.
18. Gerra G, Fertonani-Affini G, Caccavari R, Zaimovic A, Tagliavini P, Riva M, Delsignore R. Gamma-hydroxybutyric acid in the treatment of heroin addiction. New Trends Clin. Neuropharmacol 1994; 8:357–362.
19. Levy MI, Davis BM, Mohs RC, Trigos GC, Mathe AA, Davis KL. Gamma-hydroxybutyrate in the treatment of schizophrenia. Psychiatry Res 1983; 9:1–8.
20. Rinaldi F, Puca FM, Mastrosimone F, Memoli G. [On the use of gamma-hydroxybutyrate of sodium in psychiatric therapy]. Acta Neurol (Napoli) 1967; 22:21–41.
21. Otani A, Vergoni AV, Saltini S, Mioni C, Giuliani D, Bartiromo M, Zaffe D, Botticelliet AR, Ferrari A, Bertolini A, Genedani S. Effect of late treatment with γ-hydroxybutyrate on the histological and behavioral consequences of transient brain ischemia in the rat. Eur J Pharmacol 2004; 485: 183–191.
22. Mamelak M, Escriu JM, Stokan O. Sleep-inducing effects of gammahydroxybutyrate. Lancet, August 11, 1973; 328–329.
23. Scrima L, Hartman PG, Johnson FH Jr, Thomas EE, Hiller FC. The effects of gamma-hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. SLEEP 1990; 13:479–490.
24. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. SLEEP. 1991; 14:540–545.
25. U.S. Xyrem Multi-center Study Group. A randomized, double blind, placebo-controlled multi-center trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. SLEEP 2002; 25:42–49.
26. Xyrem® International Study Group. Further evidence supporting the use of sodium oxybate for the treatment of cataplexy: A double-blind, placebo-controlled study in 228 patients. Sleep Med 2005; 6:415–421.
27. U.S. Xyrem Multi-center Study Group. A 12-month, open-label, multi-center extension trial of orally administered sodium oxybate for the treatment of narcolepsy. SLEEP 2003; 26:31–35.

28. U.S. Xyrem Multi-center Study Group. Sodium oxybate demonstrates long-term efficacy for the treatment of cataplexy in patients with narcolepsy. Sleep Med 2004; 5:119–123.
29. Mamelak M, Black J, Montplaisir J, Ristanovic R. A pilot study on the effects of sodium oxybate on sleep architecture and daytime alertness in narcolepsy. SLEEP 2004; 27:1327–1334.
30. Mitler MM, Gujavarty KS, Browman CP. Maintenance of wakefulness test: a polysomnographic technique for evaluation treatment efficacy in patients with excessive somnolence. Electroencephalogr Clin Neurophysiol 1982; 53:658–661.
31. Borgen LA, Okerholm RA, Lai A, Scharf MB. The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. J Clin Pharmacol 2004; 44:253–257.
32. Scharf MB, et al. SLEEP 1998; 21:507.
33. Borgen LA, Okerholm R, Morrison D, Lai A. The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. J Clin Pharmacol 2003 Jan; 43(1):59–65.
34. Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. Prog Neurobiol 1997 Feb; 51(3):337–361.
35. Ferrara SD, Tedeschi L, Frison G, Orlando R, Mazzo M, Zordan R, Padrini R, Palatini P. Effect of moderate or severe liver dysfunction on the pharmacokinetics of gamma-hydroxybutyric acid. Eur J Clin Pharmacol 1996; 50(4):305–310.
36. Borgen LA, Lane E, Lai A. Xyrem® (sodium oxybate): Pharmacokinetic interactions with Provigil® (modafinil) in healthy human subjects. Clinical Pharmacology and Therapeutics 2001; 69(2):P17.
37. Borgen LA, Okerholm, RA, Lai A. Pharmacokinetic interactions of zolpidem and protriptyline with sodium oxybate oral solution in healthy human subjects. SLEEP 2001; 24(Suppl):A98.
38. Gibson KM, Hoffman GF, Hodson AK, Bottiglieri T, Jakobs C. 4-Hydroxybutyric acid and the clinical phenotype of succinic semialdehyde dehydrogenase deficiency, an inborn of GABA metabolism. Neuropediatrics (1998) 29:14–22.
39. Fuller DE, Hornfeldt CS. From Club Drug to Orphan Drug: Xyrem® (sodium oxybate) for the treatment of cataplexy. Pharmacotherapy 2003; 23:1205.
40. Tarabar AF, Nelson LS. The gamma-hydroxybutyrate withdrawal syndrome. Toxicol Rev 2004; 23(1):45–49.
41. ElSohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. J Anal Toxicol 1999; 23:141–146.
42. Woolverton WL, Rowlett JK, Winger G, Woods JH, Gerak LR, France CP. Evaluation of the reinforcing and discriminative stimulus effects of GHB in rhesus monkeys. Drug Alcohol Depend 1999; 54:137–143.
43. Galloway GP, Frederick SL, Staggers FE Jr, Gonzales M, Stalcup SA, Smith DE. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 1997; 92:89–96.
44. Dyer JE, Roth B, Hyma BA. Gamma-hydroxybutyrate withdrawal syndrome. Ann Emerg Med 2001; 37:147–53.
45. US Xyrem Multi-center Study Group. The abrupt cessation of therapeutically administered sodium oxybate (GHB) does not cause withdrawal symptoms. J Toxicol Clin Toxicol 2003; 41:131–135.

55
Perspectives for New Treatments: An Update

EMMANUEL MIGNOT and SEIJI NISHINO
Stanford University School of Medicine, Stanford, California, U.S.A.

Current treatments for human narcolepsy are symptomatically based, with primary effects on dopaminergic transmission for stimulants, adrenergic/serotonergic reuptake for anticataplectic antidepressants, and most likely GABA-B agonistic effects for sodium oxybate (GHB) (1–3). Thanks to renewed interest in this area, novel therapies are emerging. These can be classified into four different broad areas discussed below.

I. Improving Current Therapeutic Strategies Using Novel Antidepressants, Stimulants, and Hypnotics

At a recent meeting on translational research in sleep medicine, key recommendations in narcolepsy/hypersomnia pertained to the education of physicians on the use of novel antidepressants and stimulants in the treatment of narcolepsy. Indeed, old tricyclic antidepressants, such as clomipramine or protrityline, together with amphetamine or methylphenidate are still too often used as first intention treatments. These therapies are efficacious and not expensive, but more recent alternatives should also be considered. In the anticataplectic class, this includes compounds with adrenergic (e.g., atomoxetine) or dual adrenergic/serotonergic (e.g., venlafaxine) reuptake inhibitors that do not have anticholinergic or alpha-adrenergic effects (3,4). Modafinil, a compound with lower abuse potential and probably fewer cardiovascular effects, is also typically used as a first indication treatment (5). The mode of action of modafinil is debated but likely to involve dopaminergic reuptake inhibition and possibly other effects (2,6,7).

The current success of modafinil, with its recently extended indications to shift workers and residual sleepiness in sleep apnea (4), together with the expanding use of stimulants for attention deficit hyperactivity disorders and the need for novel treatments for resistant depression continues to fuel the growth of new products in this area (Table 1). Novel compounds likely to be developed include *L*-modafinil, the longer acting isomer of the currently available racemic modafinil mixture (8). The half-life of *L*-modafinil is approximately 3 times longer than that of *D*-modafinil in humans. This variation will increase the half-life of the product, facilitating treatment using one dose per day. A number of companies have also developed improved delivery formulations and single isomer preparations for typical stimulants such as methylphenidate and amphetamines (2).

Table 1 Possible Treatment Options for Hypocretin-Ligand Deficient and Non-Deficient Narcolepsy

Treatment type	Examples	Limitations	Notes	References
Symptomatic pharmacotherapies				
Dopaminergic and non-catecholaminergic stimulants for excessive daytime sleepiness (EDS)	Dopamine (DA) uptake inhibitors Histamine H3 antagonists TRH analogs Excitatory amino acid receptors	Wake-promoting potency of most compounds is unknown in humans	Beneficial effects of DA uptake inhibitors, H3 antagonists, TRH analogs are established in canine model of narcolepsy TRH and H3 antagonists also reduce canine cataplexy	2,4,22,24
Non-tricyclic antidepressants for cataplexy	Adrenergic reuptake inhibitors Dual (adrenergic/serotonergic) reuptake inhibitors Trimonoamine (plus dopamine) reuptake inhibitors	Compounds might be withdrawn if antidepressant effects are not prominent Potential impact on the cardiovascular system	Animal experiments suggest the importance of the adrenergic system for the mediation of anticataplectic effects Trimonoamine reuptake inhibitors may work on both cataplexy and EDS	3,4,9,10
Slow wave sleep (SWS)-enhancing hypnotics for insomnia, EDS and cataplexy	Longer half-life sodium oxybate (GHB) derivatives Gaboxadol (GABA-A agonist) Tigabine (GABA uptake blocker)	Beneficial effect of SWS enhancing hypnotics (other than GHB) on EDS and cataplexy are not known	Most probably will remain ancillary treatments for disturbed nocturnal sleep	13,14

Preventive and hypocretin-based therapies				
Hypocretin-based therapies	Peptide agonists	Limited central nervous system penetration, unstable in vivo	Intranasal administration prodrug, modification	31,32,34
	Non-peptide agonists	Not yet available Need adequate half life to reproduce natural circadian fluctuation	May be the best hope for narcoleptic patients	44,45
	Cell Transplantation	Low survival rate and limited availability of donor cells Potential re-destruction or rejection	The development of cell lines derived from embryonic or neural stem cells may provide a solution to long term problems with supply	39
	Gene therapy	Need appropriate vector; potentially dangerous side effects	Promising in the future	35
Immune-based therapies	Steroid therapies	Preventative treatment Metabolic side effects; difficult to use on a long term basis in children	Steroids work on both cell and autoantibody mediated inflammation No effect on narcolepsy in one case	53
	Intravenous-immunoglobulins (IVIg)	Preventative treatment Work on autoantibody mediated inflammation	Less invasive than plasmaphoresis Partially effective subjectively in 7 cases	56,57,58
	Plasmaphoresis	Preventative treatment Work on autoantibody mediated inflammation	More invasive than IVIg Reported partially effective in one case	60

A similar trend is also notable in the area of antidepressant therapies where novel single, dual (duloxetine, milnacipram) (9) and even triple (dopamine, serotonin, and adrenergic e.g., DOV 216,303) (10) monoaminergic reuptake inhibitors are being studied. These compounds may be of interest as modulators of both cataplexy and sleepiness, but are not genuinely novel in terms of mode of action. It is also likely that combination therapies with monoamine-selective inhibitors will often remain easier to titrate to control sleepiness and cataplexy separately.

Another potential area of improvement may be in the use of novel sedative hypnotics in narcolepsy-cataplexy. In contrast to benzodiazepine acting hypnotics, GHB has proven to be remarkably efficacious in the treatment of multiple symptoms of narcolepsy: sleepiness, cataplexy and disturbed nocturnal sleep (1,4). As discussed in other chapters, the mode of action on GHB is debated but likely involves effects on GABA-B and possibly effects on less well-characterized GHB receptors. A problem in its current formulation is the short half-life of the compound. The short half-life is an inconvenience but may improve safety and could be advantageous to avoid residual sedation and the development of tolerance. The development of longer acting GHB formulations or derivatives is ongoing. Novel GABA-B agonists or modulators may also be of interest (development is limited by epileptogenic properties at high doses), but longer acting GABA-B agonists, such as baclofen are already available and have not been systematically evaluated in human narcolepsy.

At the physiological level, GHB is unique as a strong hypnotic and because of its ability to increase deep slow wave sleep (SWS) (11). We have hypothesized that a core abnormality in hypocretin deficiency is the inability of patients to counteract even small amounts of sleep debt (12). Whether the SWS enhancing property of GHB, and the resulting decrease in homeostatic sleep debt, is needed for the beneficial effect of the compound on the various symptoms of narcolepsy is tantalizing (4). This question will only be answered when other compounds with similar SWS enhancing profile, but distinct molecular modes of action, will be available. Currently studied or available GABAergic hypnotics with SWS enhancing properties include gaboxadol, a GABAergic modulator with preferential effects on extrasynaptic GABAergic receptors containing the delta and alpha-4/5 subunits (13), and tiagabine, a GABA reuptake inhibitor (14). The existence of numerous other potential targets for hypnotics, such as 5-HT2a/c antagonists, histamine receptor antagonists, or (autoreceptor) agonists, ion channel blockers, together with the renewed interest of the pharmacological sector in hypnotic therapies may also be beneficial to narcoleptic patients. Of note, ritanserin, a 5-HT2 receptor antagonist, has been reported to have beneficial effects on disturbed nocturnal sleep in narcoleptic patients (15).

II. Non-Catecholaminergic Stimulants

The importance of histamine in sleep regulation has long been suspected considering the sedative effects of H1 receptor antagonists and the pioneering work of Lin and colleagues (16). Like adrenergic and serotonergic cells, histaminergic cells significantly decrease activity during non-REM and REM sleep (17). With regard to narcolepsy, hypocretin neurons have strong projections and excitatory effects on histamine transmission, an effect mediated by hypocretin receptor 2 (hcrtr2) (18), the receptor

mutated in canine narcolepsy. Hypocretin's effects on alertness after intracerebroventricular (icv) injections are diminished or abolished when histaminergic transmission is blocked (19), suggesting the importance of the downstream effects of hypocretin on this neurotransmitter system in mediating wake promotion. We and others also found that human narcolepsy, and possibly idiopathic hypersomnia, is associated with decreased CSF histamine (20,21). The rationale for increasing histaminergic tone to treat narcolepsy and hypersomnia is thus strong from the pathophysiological perspective.

The use of H1 receptor agonists, a logical possibility, is made impossible by the lack of available CNS penetrating compounds and due to peripheral side effects. Current industry interest is therefore mostly focused on the H3 receptor, a receptor known to be, among other actions, an autoreceptor located on histaminergic cell bodies exclusively in the brain. Stimulation of this receptor is sedative while antagonism promotes wakefulness (or reduces deep sleep) in rodents and dogs (16,22,23). Finally, experiments in narcoleptic canines have found anticataplectic and wake-promoting effects for H3 antagonists and inverse agonists (22). A significant number of pharmaceutical companies are currently developing or clinically exploring the use of H3 antagonists to promote wakefulness and cognition for various indications (e.g., JNJ-5207852) (23). Whether the promising results in animal models will also extend to humans, and whether these compounds will have enough efficacy remains to be established.

Another possible area with less current pharmaceutical interest is the use of thyrotropin-releasing hormone (TRH) direct or indirect agonists. TRH itself is a small peptide, which penetrates the blood brain barrier at very high doses. Small molecules with agonistic properties and increased blood brain barrier penetration (e.g., CG3703, CG3509 or TA0910) have been developed, partially thanks to the small nature of the starting peptide (24). Interestingly, TRH itself and TRH receptor type 2 are abundant in the reticular thalamic nucleus, where it is excitatory (25). It also has excitatory effects on motoneurons. TRH (at the high dose of several mg/kg) and TRH agonists increase alertness and have been shown to be wake promoting and anticataplectic in the narcoleptic canine model (24). Unfortunately, however, human clinical studies at low doses in depression have shown limited efficacy and only moderate subjective alerting effects (26,27); whether better compounds can and will be developed is unknown. Other possibly interesting pathways could involve inhibitors of the TRH degrading enzyme, a relatively specific metallopeptidase (28).

Other possible, still experimental targets for wake promotion could involve novel neuropeptide systems and protein targets such as circadian clock proteins or kinases, novel ion channels, prokineticin (29) or the recently described wake-promoting neuropeptide S (30).

III. Hypocretin Peptide Supplementation

The gold standard for narcolepsy treatment is likely to aim at replacing the missing dysfunctional neurotransmitter, hypocretin. This could be achieved through the delivery of hypocretin peptides themselves, through the use of prodrugs, agonists, or through the use of genetic engineering or cell replacement therapies.

Early experiments using radiolabelled hypocretin peptides had suggested that the peptide might cross the blood brain barrier by passive diffusion (31). Hypocretin-1 was

found to be more stable than hypocretin-2 in both the blood and the CSF (31,32), a property that likely explain why hypocretin-1 is more active than hypocretin-2 after icv injection. Further pharmacological experiments therefore used hypocretin-1.

A. Hypocretin Supplementation in Hcrtr2-Mutated Narcoleptic Dogs

The effects of intravenous and icv hypocretin-1 in HCRTR2-mutated canine narcolepsy was examined by John et al. (33) and Fujiki et al. (34). In 2002, John et al. found that hypocretin-1, when injected at the low dose of 3 μg/kg intravenously, was wake-promoting and able to reverse cataplexy in HRCTR2 mutated Dobermans, while 4 μg/kg administration significantly worsened cataplexy (33). This result was surprising, as in our narcoleptic HCRTR2 mutated canines, a similar dose is ineffective to produce wakefulness even when bolus intracerebroventricular (ICV) injection was made (34). A similar dose injected intravenously is also barely wake-promoting in normal dogs with functional receptors (34). Significantly higher doses were later injected both intravenously (for cataplexy and sleep) and icv (for cataplexy) without any significant effect (up to 24 μg/kg intravenously or 120 nmoles icv) in HCRTR2 mutated dogs (Fig. 1) (34), suggesting a methodological problem with the John study.

B. Hypocretin Replacement in Hypocretin-Deficient Murine and Canine Narcolepsy

A better model to assess hypocretin's effects on narcolepsy may be to use hypocretin-deficient animals. In orexin/ataxin-3 narcoleptic mice, in which hypocretin-producing neurons are ablated, icv hypocretin-1 (3 nmoles) is able to almost completely suppress episodes of behavioral arrests (cataplexy-like episodes) and to reverse sleep fragmentations and sleep onset rapid eye movement sleep periods (SOREMP) (35). These experiments strongly suggest that if delivered at the right place, hypocretin-1 could be an active treatment of narcolepsy. In two hypocretin ligand deficient canines, a close model of human narcolepsy, we administered high doses of intravenous hypocretin-1 (96–384 μg/kg) with limited effects on cataplexy (34,36). Indeed, at best, we found that the 196–384 μg/kg doses decreased cataplexy, but for less than 15 minutes (Fig. 1) (34). Whether this transient effect is a possible reflection of side effects rather than genuine therapeutic relief due to centrally penetrating hypocretin-1 is unknown. We also examined blood and CSF hypocretin-1 levels after intravenous administration and found extremely high concentration increases in the blood (up to 10 million pg/ml in blood after 384 μg/kg) with minimal and variable increase in CSF hypocretin-1 (+400 pg/ml after 384 μg/kg; with exclusion of CSF samples containing blood) (Fig. 2) (34). These results indicate that the blood brain barrier is, in fact, quite impermeable to hypocretin-1. Peripherally administered hypocretins are not likely to be effective in the treatment of narcolepsy, unless administered at much higher doses. The effects of even higher doses, similar to those found to be active for TRH (500-several mg/kg) in the canine model (24), may however still need to be tested, as no significant peripheral side effects were noted.

C. Intranasal Administration

Another possible path toward delivering hypocretin-1 to the brain may include intranasal delivery (37). In mice, we found that after intranasal ^{125}I-hypocretin 1

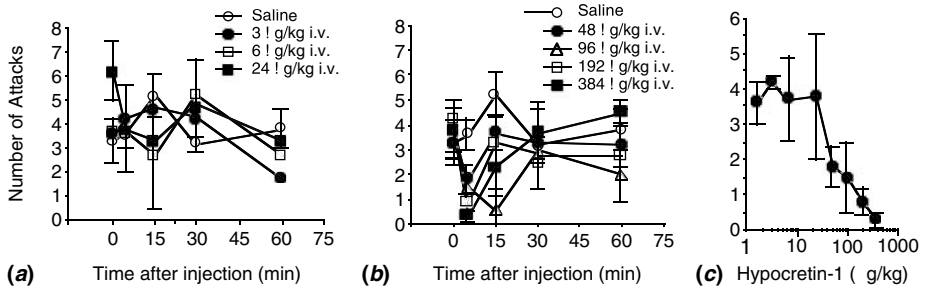


Figure 1 Effect of intravenous hypocretin-1 on cataplexy in a sporadic narcoleptic dog. After the baseline session of food elicited cataplexy test (FECT), saline or hypocretin-1 at doses of 3, 6, 24, 48, 96, 192, or 384 μg/kg were administered intravenously. Effects on cataplexy were evaluated at 5, 15, 30, and 60 minutes after the injection. Experiments were repeated three times for each dose, and the mean results of the three sessions are displayed. Time-course effects on cataplexy are shown in panel (***a***) (low doses) and (***b***) (high doses). Intravenous administration of hypocretin-1 with high doses (over 24 μg/kg) induced a very short-lasting anticataplectic effect. Numbers of attacks five minutes after the injection of each dose were replotted in panel (***c***). The suppression of cataplexy was dose dependent and in 2 out of 3 sessions that administered 384 μg/kg, cataplexy was completely suppressed. *Source*: From Ref. 34.

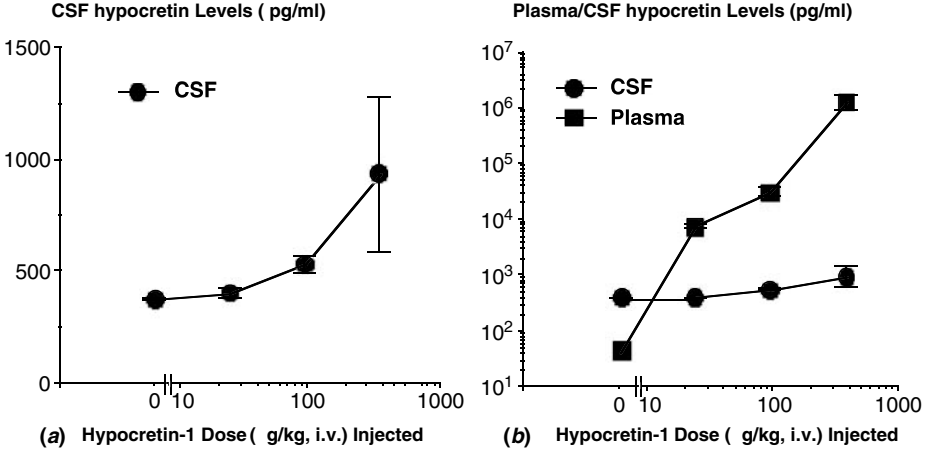


Figure 2 Central penetration of hypocretin-1. Changes in hypocretin levels in the cerebrospinal fluid (CSF) (***a***) and blood (plasma) (***b***) of two narcoleptic (HCRTR2 mutated) Dobermans were measured after intravenous (i.v.) administrations of hypocretin-1. The dogs were injected with saline and 24 μg/kg, 96 μg/kg, and 386 μg/kg of hypocretin-1. Cisternal CSF taps and blood collections were carried out 30 minutes after injection. The CSF hypocretin levels are replotted together with plasma levels using a log scale on the right panel (***b***). *Source*: From Ref. 34.

administration (5 nmoles), high levels of putative, labeled hypocretin-1 were found in the nuclei of the trigeminal nerve (estimated concentration 1,007 nM), olfactory bulbs (324 nM), and anterior olfactory nucleus (126 nM). Concentrations ranged from 14 nM to 61 nM in other regions of the brain (e.g., cortex, hippocampus, brain stem), with the highest concentration (61 nM) observed in the hypothalamus (37). In addition to delivery to the brain, intranasal hypocretin-1 also resulted in delivery to the spinal cord, with a decreasing gradient from cervical (96 nM) to lumbar (3 nM) regions. More disappointingly, however, a preliminary observation did not allow us to observe significant changes in locomotion in both control and narcoleptic mice after intranasal administration (J. Zeitzer, personal communication). Experiments in humans may be needed to further test this hypothesis.

D. Intracisternal Administration

The need for intracerebral administration finally led us to implant a time-release Medtronic pump in a 3 year-old hypocretin deficient Weimaraner with a connection to the cisterna magna (36). These pumps are commonly used to perfuse analgesic (e.g., opioids for pain) or spasmolytic (e.g., for example baclofen to treat spasticity in multiple sclerosis) compounds. Timing of the administration can be remotely controlled. It was our hope that hypocretin-1 would backflow through the foramen ovale into the cerebral ventricular system, and that a similar device could be used afterwards in humans with catheterization of the lumbar sac. These experiments were unsuccessful, even when up to 1200 nmoles of hypocretin-1 (150 μg/kg) were injected, suggesting the impracticality of this approach. Another possibility may be that hypocretin receptors are inactivated after long periods of hypocretin deficiency and thus could not be stimulated by the perfused hypocretin-1. This is however less likely, since neither hcrtr1 nor hcrtr2 mRNA were found to be significantly decreased in hypocretin deficient human brains (M. Honda, unpublished results using human cDNA arrays in postmortem human narcolepsy brains). We are nonetheless planning to pursue this experimental approach with direct lateral ventricle perfusion of hypocretin-1 the next time any hypocretin deficient dogs are identified.

E. Gene Therapy

The last possible experimental approach using native hypocretins could involve gene therapy or hypocretin cell replacement therapy. Indeed, Mieda et al., using mice non-specifically over-expressing the hypocretin gene in the central nervous system using a beta-actin/cytomegalovirus hybrid promoter found that crossing these mice with orexin/atxin-3 narcoleptic mice was able to rescue the phenotype of narcolepsy (both sleep abnormalities and behavioral arrests) (35). It is therefore theoretically possible that viral delivery of a transgene (or indirect delivery via cells carrying such a transgene but entering the CNS) allowing the expression of hypocretin without proper anatomical distribution or physiological regulation could be effective.

F. Cell Transplantation

More promising on a medium-term basis may be the use of cell transplantation. Transplanted cells are likely to keep their regulatory mechanisms intact. Transplantation of fetal hypothalamic tissue has, for example, been shown to rescue circadian

abnormalities in suprachiasmatic nuclei (SCN) lesioned or clock mutated animals. In Parkinson disease, a large number of animal studies have indicated feasbility of cell transplantation, while clinical studies have had variable effects (38). Arias-Carrión et al. recently found that the transplantation of neonatal rat hypothalami into the brainstem of adult rats might result in the development of stable grafts containing hypocretin neurons (39). Whether these grafts will restore function and project to their normal targets is unknown.

In humans, it is estimated that about 70,000 hypocretin neurons exist in the brain and that narcolepsy is associated with a 85–95% loss (40). Although the number of neurons needed to be restored to rescue the narcolepsy phenotype is unknown, narcolepsy typically presents when CSF hypocretin-1 levels are below one third of control values (41). It is thus likely that restoring a minimum of 10% of the normal cell population may be needed to have a clinical effect.

In Parkison's disease models however, the survival rate of dopamine neurons of fetal mesencephalon grafts is only 5–10% (42). The low survival rate suggests that it may be impossible to gather enough material from cadaver donors. To solve the problem of supply, efficient cell sorting/selecting methods for hypocretin containing cells may need to be developed. A similar problem is also evident in the field of type I diabetes, where islet cell transplantation in the liver is effective but donor material is scarce and long term benefits are still unclear (43).

The possibility of immune reactions to the grafted hypocretin cells may be another concern, especially if an autoimmune process causes hypocretin-deficiency in humans. A similar problem is emerging in the area of islet cell transplantation (43). The long-term solution for these problems may therefore be the genetic engineering of cells delivering hypocretins, either using stem cell technology or genetically modified transplanted cells. In this area like in others, narcolepsy is likely to benefit from parallel advances in other areas of medicine.

G. Hypocretin Peptide Analogues

An obvious solution considering the lack of CNS penetration of hypocretin peptides themselves may be to develop centrally acting hypocretin agonists. The molecular size and innate water-solubility of compounds are some of the important issues to consider when attempting to deliver peptides effectively into the brain parenchyma. The fact that most of the narcolepsy phenotype is recapitulated by HCRTR2 deficient animals suggests that HCRTR2 rather than HCRTR1 agonists may be most appropriate. Hypocretin-2, a peptide with higher HCRTR2 versus HCRTR1 affinity, may be useful starting point, but it has very short biological half-live (31,32); more stable molecular entities are desired for pharmacological delivery.

The modification of the native peptide or the design of precursor molecules (*i.e.*, prodrug) may potentially overcome these hurdles. Substitution scans, truncated peptide analysis and cross-species comparisons indicate that the C-terminal amide portion of both hypocretin peptides, most notably the 8 last amino acids, a region of high homology between hypocretin-1 and 2 is most critical (44,45). Selected identified peptide substitutions were also found to have increased selectivity toward the HCRTR1 and HCRTR2 receptors (44,45). Unfortunately, however, none of the provided structures are small enough to be likely drugs, although the study of these modified peptides at

very high doses, together with further structural improvement to increase stability/central penetration may still be a viable research direction.

H. Hypocretin Agonists

Direct agonists with adequate pharmacokinetic properties would be ideal therapies. For most G-protein coupled receptor (GPCR) systems, however, it is typically more difficult to identify agonists than antagonists. Indeed, agonists must not only bind the receptor, but must also interact tighly at the molecular level to stimulate secondary messenger systems. This may even be more dificult for peptide receptor systems, considering the size of the ligand and the potential complexity of the associated molecular interactions. In spite of these difficulties, a dozen non-peptide agonists for GPCR peptide receptors, including urotensin-II receptor (GPR14) (46), opioid receptor-like (ORL1) receptor (47), and galanin receptor (48) are currently under development.

Several companies have succeeded in the identification of small molecule hypocretin receptor antagonists (49–52), both with some hcrtr1 and hcrtr2 selectivity. These molecules are active *in vitro* and *in vivo*, but it is still too early to predict whether some of these compounds have the right properties to become viable drugs. It is also unclear if these drugs will have an adequate side-effect profile and if a proper indication will be found inside or outside the sleep disorder market. Whether non-peptide hypocretin receptor agonists can or will be identified and successfully developed is unpredictable.

IV. Immunomodulation as a Preventive Treatment for Narcolepsy

The combined observation of hypocretin cell loss and HLA association suggests an autoimmune basis for narcolepsy. If this is the case, however, it is likely that the process is only reversible prior to near complete ablation of cells. Studies in young children close to an abrupt onset (typically 3 months-1 year) indicate that in most cases, CSF hypocretin-1 is undetectable or very low at the time of presentation, even when the subject does not have cataplexy (53,54). This unfortunately suggests that symptoms only appear once the majority of the cell population is destroyed. Indeed, rat studies also indicate that a 70% cell loss only results in a 50% decrease in CSF hypocretin-1, suggesting compensation in cases of partial cell loss (55). It is however also possible that the loss CSF hypocretin-1 is a reflection of decreased cell function without actual cell death and that the destruction can still be partially reversed at this early stage.

To address this issue, we first attempted to reverse narcolepsy using prednisone in an 8-year-old child with abrupt onset 3 months prior to diagnosis (53). The child had already undetectable CSF hypocretin-1, no cataplexy and a positive multiple sleep latency test (MSLT). Prednisone was selected as a broad cell mediated and antibody mediated immunosuppressant. Repeat MSLT and CSF evaluation was performed after 3 weeks, and no clinical improvements were noted. This child now has developed cataplexy and symptoms are controlled by venlafaxine and modafinil. A similar prednisone trial was also performed in a hypocretin deficient narcoleptic dog (Weimaraner) diagnosed 2 weeks after an abrupt onset, with similar negative results (36).

In a recent onset patient with cataplexy, combining intravenous immunoglobulins (IVIg) and prednisone reduced cataplexy and sleepiness subjectively, but the patient was unable to continue treatment (56). Dauvilliers et al. (57) and Zuberi et al. (58), studied four additional patients each with IVIg alone; six with recent onset, two with more distant disease onset. Four monthly treatments using 2 g/kg over 2 days were typically performed. Subjective effects on sleepiness and/or cataplexy were observed in recent onset cases but not in established narcolepsy cases (onset more than a few years old). Most notably, in the Dauvilliers study (57), cataplexy was very reduced and anticataplectic drugs were not needed even six months after ending the IVIg treatment, suggesting long-term preventive effects.

Problematically, however, all the reported effects were subjective. Repeat CSF hypocretin-1 measurements in the Dauvilliers study (57) indicated persistence of low CSF hypocretin-1, although in one case a possible small increase (from undetectable to 79 pg/ml) was noted. Whether a small reduction of hypocretin deficiency, without increasing CSF hypocretin-1 levels above the limit of detection is present, may have to be addressed by improving sensitivity of the reported measurements. Similarly, reports by Zuberi indicated more improvement in subjective sleepiness than in cataplexy (58). Finally, MSLT and maintenance of wakefulness test (MWT) evaluation in the Dauvilliers study indicated a non-significant small improvement in sleep latency in two cases, but not a resolution of the phenotype. Indeed, SOREMPs were still observed in all situations.

To confirm these results using proper double blind, placebo-controlled trial may be needed. The report of narcolepsy within a few month of onset is a rare occurrence, and pooling resources will be necessary. It may also be interesting to explore the effect of other immunomodulatory treatments, for example those acting on TNF, B cells, or other lymphokines. Finally, the mode of action of IVIg on these symptoms will need to be studied further. IVIg are frequently believed to act by clearing autoantibodies, yet most attempts at detecting such pathogenic antibodies in human narcolepsy have failed (59). The IVIg mixture is complex and modulation of other arms of the immune system is involved. To address this issue, studying the effect of plasmaphoresis, another treatment for antibody-mediated diseases may provide some answers. We recently reported a case where plasmaphoresis had some temporary efficacy in an adult with unusually severe and late onset at age 60 (60). Whether immune modulation close to the onset, and associated preventive measures, such as the monitoring of children at risk (e.g., family members with the disease HLA haplotype), as performed in Scandinavia with Type I diabetes, will one day be done, remains far in the future.

In conclusion, the treatment of human narcolepsy is rapidly evolving. Much progress has been made recently through the improvement of currently available symptomatically based, monoaminergic therapies. Novel stimulants and hypnotics are being developed and may benefit narcoleptic patients. More excitingly however, hypocretin replacement therapies are being designed. The most promising avenues include hypocretin agonists and hypocretin cell transplantation therapies, but these modalities are most likely decades away. Recent results using IVIg, although preliminary, also suggest the possibility of early intervention to limit disease progression, if associated with early diagnosis close to disease onset.

Acknowledgment

This work was supported by NIH NS 23724, NS33797 and HL59601 to E. Mignot. E. Mignot is a Howard Hughes Medical Investigator.

References

1. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Progress in Neurobiology 1997; 52:27–78.
2. Nishino S, Mignot E. CNS stimulants, In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. Philadelphia: W. B. Saunders Company, 2005: in press.
3. Nishino S. Anticataplectic medications. In: Narcolepsy and Hypersomnia, Bassetti C, Billiard M, Mignot E, eds. Marcel Dekker, this volume.
4. Mignot E. An update on the pharmacotherapy of excessive daytime sleepiness and cataplexy. Sleep Medicine Reviews 2004; 8(5):333–338.
5. US Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology 2000; 54:1166–1175.
6. Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. Journal of Neuroscience 2001; 21:1787–1794.
7. Saper CB, Scammell TE. Modafinil: A drug in search of a mechanism. Sleep 2004; 27:11–12.
8. Donovan JL, Malcolm RJ, Markowitz JS, DeVane CL. Chiral analysis of d- and l-modafinil in human serum: application to human pharmacokinetic studies. Therapeutic Drug Monitoring 2003; 25:197–202.
9. Kent JM. SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. Lancet 2000; 355(9207):911–918. Erratum in: Lancet 2000; 355(9219):2000.
10. Beer B, Stark J, Krieter P, Czobor P, Beer G, Lippa A, Skolnick P. DOV 216,303, a "triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile. Journal of Clinical Pharmacology 2004; 44(12):1360–1367.
11. Scrima L, Hartman PG, Johnson Jr. FH, Thomas EE, Hiller FC. The effects of gamma-hydroxybutyrate on the sleep of narcolepsy patients: a double-blind study. Sleep 1990; 13:479–490.
12. Zeitzer JM, Mignot E. In: Narcolepsy and Hypersomnia, Bassetti C, Billiard M, Mignot E, eds. Marcel Dekker, this volume.
13. Krogsgaard-Larsen P, Frolund B, Liljefors T, Ebert B. GABA(A) agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. Biochemistry and Pharmacology 2004; 68:1573–1580.
14. Mathias S, Wetter TC, Steiger A, Lancel M. The GABA uptake inhibitor tiagabine promotes slow wave sleep in normal elderly subjects. Neurobiology of Aging 2001; 22(2):247–253.
15. Mayer G. Ritanserin improves sleep quality in narcolepsy. Pharmacopsychiatry 2003; 36(4):150–155.
16. Lin JS. Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. Sleep Medicine Reviews 2000; 4:471–503.
17. Steininger TL, Alam MN, Gong H, Szymusiak R, McGinty D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. Brain Research 1999; 840:138–147.
18. Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, et al. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. Neuron 2003; 38:715–730.
19. Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, et al. Arousal effect of orexin A depends on activation of the histaminergic system. Proceedings of the National Academy of Sciences USA 2001; 98:9965–70.
20. Kanbayashi T, Kodama T, Hondo H, et al. CSF histamine and noradrenaline contents in narcolepsy and other sleep disorders. Sleep 2004; 27:A236.
21. Nishino S, Sakurai E, Nevisimalova S, et al. CSF histamine content is decreased in hypocretin-deficient human narcolepsy. Sleep 2002; 25:A476.
22. Tedford CE, Edgar DM, Seidel WF, et al. Effects of a novel, selective, and potent histamine H3 receptor antagonist, GT-2332, on rat sleep/wakefulness and canine cataplexy. Society for Neuroscience Abstracts 1999; 25:1134.

23. Barbier AJ, Berridge C, Dugovic C, Laposky AD, Wilson SJ, Boggs J et al. Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H3 antagonist. British Journal of Pharmacology 2004; 143(5):649–661.
24. Nishino S, Arrigoni J, Shelton J, Kanbayashi T, Dement WC, Mignot E. Effects of thyrotropin-releasing hormone and its analogs on daytime sleepiness and cataplexy in canine narcolepsy. Journal of Neuroscience 1997; 17:6401–6408.
25. Heuer H, Schafer MK, O'Donnell D, Walker P, Bauer K. Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. Journal of Comparative Neurology 2000; 428:319–336.
26. Vogel HP, Benkert O, Illig R, Muller-Oerlinghausen B, Poppenberg A. Psychoendocrinological and therapeutic effects of TRH in depression. Acta Psychiatrica Scandinavica 1977; 56:223–232.
27. Bunevicius R, Matulevicius V. Short-lasting behavioural effects of thyrotropin-releasing hormone in depressed women: results of placebo-controlled study. Psychoneuroendocrinology 1993; 18:445–449.
28. Schomburg L, Turwitt S, Prescher G, Lohmann D, Horsthemke B, Bauer K. Human TRH-degrading ectoenzyme cDNA cloning, functional expression, genomic structure and chromosomal assignment. European Journal of Biochemistry 1999; 265:415–422.
29. Mignot E, Taheri S, Nishino S. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nature Neuroscience 2002; 5S:1–6
30. Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004; 43:487–497.
31. Kastin AJ, Akerstrom V. Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. Journal of Pharmacology and Expermental Therapeutics 1999; 289:219–223.
32. Yoshida Y, Fujiki N, Maki RA, Schwarz D, Nishino S. Differential kinetics of hypocretins in the cerebrospinal fluid after intracerebroventricular administration in rats. Neuroscience Letters 2003; 346:182–186.
33. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Research Online 2000; 3:23–28.
34. Fujiki N, Yoshida Y, Ripley B, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin ligand deficient narcoleptic dog. Sleep 2003; 6:953–959.
35. Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proceedings of the National Academy of Sciences USA 2004; 101(13):4649–4654.
36. Schatzberg SJ, Cutter-Schatzberg K, Nydam D, Barrett J, Penn R, Flanders J, et al. Effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. Journal of Veterinary Internal Medicine 2004; 18:586–588.
37. Hanson LR, Martinez PM, Taheri S, Kamsheh L, Mignot E, Frey II WH. Intranasal administration of hypocretin 1 (orexin A) bypasses the blood-brain barrier & targets the brain: a new strategy for the treatment of narcolepsy. Drug Delivery Technology 2004; 4:66–71.
38. Roitberg B, Urbaniak K, Emborg M. Cell transplantation for Parkinson's disease. Neurology Research 2004; 26:355–362.
39. Arias-Carrion O, Murillo-Rodriguez E, Xu M, et al. Transplant of hypocretin neurons into the pontine reticular formation: Preliminary results. Sleep 2004: in press.
40. Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27:469–474.
41. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Archives of Neurology 2002; 59:1553–1562.
42. Bjorklund A. Neurobiology. Better cells for brain repair. Nature 1993; 362:414–415.
43. Couzin J. Diabetes. Islet transplants face test of time. Science 2004; 306(5693):34–37.
44. Asahi S, Egashira S, Matsuda M, Iwaasa H, Kanatani A, Ohkubo M, et al. Development of an orexin-2 receptor selective agonist, [Ala(11), D-Leu(15)]orexin-B. Bioorganic and Medicinal Chemistry Letters 2003; 13:111–113.
45. Darker JG, Porter RA, Eggleston DS, Smart D, Brough SJ, Sabido-David C, Jerman JC. Structure-activity analysis of truncated orexin-A analogues at the orexin-1 receptor. Bioorganic and Medicinal Chemistry Letters 2001; 11:737–740.

46. Croston GE, Olsson R, Currier EA, Burstein ES, Weiner D, Nash N, et al. Discovery of the first non-peptide agonist of the GPR14/urotensin-II receptor: 3-(4-chlorophenyl)-3-(2- (dimethylamino)ethyl)isochroman-1-one (AC-7954). Journal of Medicinal Chemistry 2002; 45:4950–4953.
47. Zaveri N. Peptide and nonpeptide ligands for the nociceptin/orphanin FQ receptor ORL1: research tools and potential therapeutic agents. Life Sciences 2003; 73:663–678.
48. Saar K, Mazarati AM, Mahlapuu R, Hallnemo G, Soomets U, Kilk K, et al. Anticonvulsant activity of a nonpeptide galanin receptor agonist. Proceedings of the National Academy of Sciences USA 2002; 99:7136–7141.
49. Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA, Jerman JC. SB-334867-A: the first selective orexin-1 receptor antagonist. British Journal of Pharmacology 2001; 132:1179–1182.
50. Porter RA, Chan WN, Coulton S, Johns A, Hadley MS, Widdowson K, et al. 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. Bioorganic and Medicinal Chemistry Letters 2001; 11:1907–1910.
51. Langmead CJ, Jerman JC, Brough SJ, Scott C, Porter RA, Herdon HJ. Characterisation of the binding of [3H]-SB-674042, a novel nonpeptide antagonist, to the human orexin-1 receptor. British Journal of Pharmacology 2004; 141:340–346.
52. Hirose M, Egashira S, Goto Y, Hashihayata T, Ohtake N, Iwaasa H, et al. N-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline: the first orexin-2 receptor selective non-peptidic antagonist. Bioorganic and Medicinal Chemistry Letters 2003; 13:4497–4499.
53. Hecht M, Lin L, Kushida CA, Umetsu DT, Taheri S, Einen M, Mignot E. Report of a case of immunosuppression with prednisone in an 8-year-old boy with an acute onset of hypocretin-deficiency narcolepsy. Sleep 2003; 26:809–810.
54. Arii J, Kanbayashi T, Tanabe Y, et al. SF hypocretin-1 (orexin-A) levels in childhood narcolepsy and neurologic disorders. Neurology 2004: in press.
55. Gerashchenko D, Murillo-Rodriguez E, Lin L, Xu M, Hallett L, Nishino S, et al. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Experimental Neurology 2003; 184:1010–1016.
56. Lecendreux M, Maret S, Bassetti C, Mouren MC, Tafti M. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old boy. Journal of Sleep Research 2003; 12(4):347–348.
57. Dauvilliers Y, Carlander B, Touchon J, Tafti M. Successful management of cataplexy with intravenous immunoglobulins at narcolepsy onset. Annals of Neurology 2004; 56(6):905–908.
58. Zuberi SM, Mignot E, Ling L, et al. Variable response to intravenous immunoglobulin therapy in childhood narcolepsy. Journal of Sleep Research 2004; 13(Suppl. 1):828.
59. Taheri S, Krempetz M, Jackson M, et al. Autoimmune basis of narcolepsy using western blot analysis of lateral hypothalamus protein extract with serum and cerebrospinal fluid (CSF). Sleep 2003; 26:A285.
60. Chen W, Black J, Einen M, Mignot E. Late onset narcolepsy presenting as rapidly progressing muscle weakness: response to plasmaphoresis. Ann Neurol 2005 Sep; 58(3):489–490.

56
Diagnostic Criteria for Syndromes of Excessive Daytime Sleepiness

EMMANUEL MIGNOT
Stanford University School of Medicine, Stanford, California, U.S.A.

MICHEL BILLIARD
Gui de Chauliac Hospital, Montpellier, France

CLAUDIO L. BASSETTI
University Hospital, Zurich, Switzerland

I. Introduction

A revision of the International Classification of Sleep Disorders (ICSD) has just been completed (1). In the previous classification published in 1990, and revised in 1997 (2), narcolepsy and idiopathic hypersomnia were distinguished. Other conditions listed included recurrent hypersomnia (such as the Kleine-Levin syndrome), posttraumatic hypersomnia (all part of a section called intrinsic sleep disorders), and insufficient sleep syndrome (part of a section called extrinsic sleep disorders).

The architecture of the old ICSD-1 classification (2), based on distinguishing "dysomnias" (intrinsic and extrinsic disorders), "parasomnias", and "medical/psychiatric disorders" was abandoned. In the revised ICSD-2, the classification architecture is primarily based on the major presenting symptom. As such, disorders with excessive daytime sleepiness were all included in a new section called "Hypersomnia not due to a Sleep Related Breathing Disorder". The revised classification described below was the result of a 2-year process. The key actors in this process were participants to the task force working on narcolepsy and hypersomnia (Table 1). Most of the work was done through e-mail correspondence, but the committee also met once. This task force included the authors and 12 specialists from seven countries total (Table 1). Representative from most of the major groups with clinical and research experience in hypersomnia were represented. A pediatric neurologist with experience in this area was also included (SK). Evidence-based medicine (publication reviews) was first used, followed by consensus decision by the task force if necessary (Table 1). The task force was instructed not to change the names of established conditions unless absolutely necessary. The final classification and approved diagnostic criteria were next reviewed by the ICSD-2 steering committee (composed of the chairs of all sections and Dr. Peter Hauri). The American Academy of Sleep Medicine (AASM) board of directors finally reviewed the document and provided additional feedback. Editorial assistance was provided by the AASM.

Table 1 Participants to the ICSD-2 Task Force, Hypersomnia Section

Participant	Affiliation	Country
Emmanuel Mignot. M.D., Ph.D., Chair of Hypersomnia Task Force, ICSD-2	Stanford University, Palo Alto, California	United States
Claudio Bassetti, M.D.	Neurologische Poliklinik, Zurich	Switzerland
Michel Billiard. M.D.	Montpellier University, Montpellier	France
Roger Broughton, M.D., Ph.D.	University of Ottawa, Ottawa	Canada
Ronald Chervin, M.D., M.S.	University of Michigan, Ann Arbor, Michigan	United States
Christian Guilleminault, M.D., Ph.D.	Stanford University, Palo Alto, California	United States
Yutaka Honda,, M.D., Ph.D.	Seiwa Hospital, Tokyo	Japan
Takashi Kanbayashi, M.D.	Akita University, Akita	Japan
Suresh Kotagal, M.D.	Mayo Clinic, Rochester, Minnesota	United States
Gert Jan Lammers, M.D, Ph.D.	Leiden University, Leiden	The Netherlands
Sona Nevsimalova, M.D., Ph. D.	Charles University, Prague	Czech Republic
Seiji Nishino, M.D., Ph.D.	Stanford University, Palo Alto, California	United States
Thomas Scammel, M.D., Ph.D.	Harvard University	United States
Michael Silber, M.B, Ch.B	Mayo Clinic, Rochester, Minnesota	United States
Michael Thorpy, M.D., chair of Diagnostic classification steering committee for the ICSD-1	Albert Einstein College of Medicine, Bronx, New York	United States

For details, see ICSD-2 (1).

It should be noted that the process was difficult. Indeed, task force consensus, sometimes obtained after much deliberation, was not always accepted during further reviews by the ICSD-2 steering committee or the AASM board of directors. Examples of controversial issues included the naming of the conditions, the use of "variants" of "subtypes" to define some of the subcategories and the need to use the MSLT for various diagnostic entities (and with what cut-off mean sleep latency values). The revised architecture and diagnostic criteria, proposed below, were the result of a consensus decision, and do not necessarily reflect the opinion of the authors above.

In the revised version, narcolepsy was separated into narcolepsy with and without cataplexy. Idiopathic hypersomnia was separated into idiopathic hypersomnia with and without long sleep time (Table 2). The Multiple Sleep Latency Test (MSLT), a test established in the 1970s, gained importance in the revised classification as the test of choice to classify these disorders, reflecting current practice in the United States, Europe and increasingly, all over the world (3,4).

In the use of this test, the observation of more than 2 sleep onset REM periods (SOREMPs) was considered diagnostic for narcolepsy. A mean sleep latency equal or below 8 minutes was used to objectively define daytime sleepiness. This cut off value (rather than a 5 minute cut off) was used based on data from Moskovitch et al. (5) and Aldrich et al. (6) comparing narcolepsy versus other disorders. The same cut off was expanded to idiopathic hypersomnia for reason of simplicity.

Other minor changes were made. Post-traumatic hypersomnia was deleted, and instead separate sections were created to reflect the existence of secondary narcolepsy and secondary hypersomnia. Recurrent hypersomnia was also modified to include not only monosymptomatic and polysymptomatic Kleine-Levin syndrome but also the rare menstrual associated sleep disorder.

II. On the Use and the Misuses of the Multiple Sleep Latency Test (MSLT)

The growing importance of the MSLT in diagnosing these conditions led the committee to reflect on the value of this test. Limited population-based studies are available, thus normative data are not well established. Mean sleep latency measurements on the MSLT are affected by sleep deprivation (and insufficient sleep, shift work) (7,8), sleep disorders (e.g. abnormal breathing during sleep, circadian disorders) (6,9) and psychotropic medications (3). Little is known regarding what could influence SOREMPs but the same factors are suggested to be involved (7,8). It was also noted that a very large portion of the population may have a mean sleep latency below 8 minutes (4). Finally, the fact that the test has never been validated in young children, even to diagnose narcolepsy, was discussed.

In an attempt to resolve these issues, a strong introduction to the overall hypersomnia section was written to reflect these unknowns (1). Practitioners were encouraged not to use the MSLT without careful consideration of the above and only in correlation with the clinical picture. Most notably, it was suggested that all patients be well rested before MSLTs, as documented by a one weeklong sleep log. Similarly, the MSLT was considered "suspicious" if total sleep time during nocturnal sleep recording prior to the MSLT was below 6 hrs or if significant sleep disordered breathing (or any other disorder causing sleep disruption) was present. In the case of sleep apnea, it was advised to first treat the condition and to repeat the MSLT only if sleepiness persisted in the face of adequate treatment/compliance for this condition. MSLT SOREMPs were considered impossible to predict if patients had been on psychotropic drugs shortly before (2 week wash out needed after antidepressants) or were currently on psychotropic medications. Finally, attention must be paid to the possibility of delayed sleep phase or shift work interfering with the test.

Of note, many of these caveats reflect common sense but have not been validated by any formal study. The suggestions proposed above are also difficult to implement and may be commonly ignored in some diagnostic centers. This may be especially problematic for idiopathic hypersomnia without long sleep time, a condition characterized by a complaint of sleepiness and a mean sleep latency below or equal to 8 minutes on the MSLT but less than 2 SOREMPs. Indeed, limited population based data suggest that a large portion of the population, if tested randomly, may well fit diagnostic criteria for this condition (4), for example if only mildly sleep deprived.

III. Narcolepsy with and Without Cataplexy

The rationale for separating narcolepsy with and without cataplexy was the result of philosophy, recent pathophysiological findings and of differences in practice between

Table 2 International Classification of Sleep Disorders (ICSD-2): Definitions, Prevalence and Pathophysiology of Narcolepsy and Hypersomnia

Condition	Diagnostic Criteria	Prevalence	Pathophysiology
Narcolepsy with cataplexy	EDS; Presence of definite cataplexy; usually abnormal MSLT results	0.02 to 0.067 percent	Hypocretin deficiency; 90 percent with low CSF hcrt-1 and positive for HLA-DQB1*0602
Narcolepsy without cataplexy	EDS; No or doubtful cataplexy; MSLT: MSL≤8 minutes,≥2 SOREMPs	0.02 percent if diagnosed Possibly common, several percent if undiagnosed	Unknown, probably heterogeneous; 19 percent with low CSF hcrt-1, 40 percent HLA-DQB1*0602 positive
Idiopathic hypersomnia-with long sleep time	EDS; long (10 hours or greater) unrefreshing nocturnal sleep; MSLT: typically but not always MSL≤8 minutes,<2 SOREMPs	Rare, maybe 0.01 to 0.02 percent	Unknown, probable heterogeneous etiology
Idiopathic hypersomnia-without long sleep time (normal sleep length)	EDS; normal nightly sleep amounts (> 10 hours); MSLT: MSL≤8 minutes,<2 SOREMPs	Possibly common, unknown prevalence	Unknown, probable heterogeneous etiology
Secondary Narcolepsy	As above, but due to other known medical conditions (e.g., neurological, drug of abuse, medication, psychiatric)	Unknown	With or without hypocretin deficiency
Periodic hypersomnia (includes Kleine-Levin syndrome and menstrual associated sleep disorder)	Recurrent (more than 1 time per year) sleepiness (lasting 2 to 28 days); normal function between occurrences	Rare, probably less than one per one million people	Unknown, probable heterogeneous etiology
Behaviorally induced insufficient sleep syndrome	EDS; short habitual sleep leading to sleep deprivation	Probably common, unknown prevalence	Chronic sleep deprivation; may be difficult to distinguish from idiopathic hypersomnia in long sleepers

For details, see ICSD-2. EDS: complaint of excessive daytime sleepiness of at least of 3 months in duration; CSF: lumbar sac cerebrospinal fluid; hcrt: hypocretin; HLA: human leukocyte antigen; MSLT: multiple sleep latency test; MSL; mean sleep latency; SOREMP: sleep-onset rapid eye movement period.

the United States and other countries. As in the past, many participants argued that "narcolepsy" should be reserved only to cases with cataplexy. As most cases with cataplexy are known to be HLA-DQB1*0602 and hypocretin deficient (10), narcolepsy as defined was likely to represent a homogenous group of well defined patients.

Others suggested a slightly more relaxed version according to which recent onset cases without cataplexy, or patients with hypocretin deficiency could be included in the "narcolepsy" section. A third group, mostly of United States origin, finally pointed out that the current use of "narcolepsy" was broader in scope, and that many patients were only diagnosed based on the report of multiple SOREMPs on the MSLT. To change their named diagnosis to "idiopathic hypersomnia", or to use "essential hypersomnia", as used in the work of Honda et al. (11), would create major confusion if not harm. It was finally argued that the cause of narcolepsy without cataplexy was unknown, as it may or may not be similar to that of narcolepsy without cataplexy based on current genetic and pathophysiological findings.

To accommodate these divergent points of view, narcolepsy with and without cataplexy were separated (Table 2). A strict definition of cataplexy in narcolepsy-cataplexy was provided, with the need for typical triggers and reasonable severity. As before, the MSLT was not considered needed for diagnosis if definite cataplexy was present. The MSLT was however highly recommended for objective documentation. Using these criteria, patients with cataplexy and an established diagnosis but no prior MSLT do not need to go through the personal expense of a drug withdrawal for MSLT testing. It was also felt that distinguishing patients with and without cataplexy was useful in the consideration of future therapies. Patients with cataplexy have a better-codified treatment path, and are better known to benefit from treatments that are delicate to prescribe because of their abuse potential, such as amphetamine and sodium oxybate.

In the revised classification, narcolepsy without cataplexy is primarily defined based on the MSLT (Table 2). Cataplexy may be present if atypical or extremely mild or doubtful. Based on previous studies using narcolepsy-cataplexy patients, a mean sleep latency less or equal to 8 minutes and more than 2 SOREMPs is required to diagnose narcolepsy without cataplexy. The presence of other REM related symptoms, hypnagogic hallucinations and sleep paralysis, in the diagnostic criteria was eliminated. The rationale for this change was the fact these symptoms have a poor predictability for narcolepsy. Indeed, these symptoms are present in controls and in subjects with other disorders (6) and are often difficult to define as pathological. As defined however, narcolepsy without cataplexy may be a heterogeneous condition that includes patients with hypocretin deficiency who have not yet developed cataplexy (or will never develop this symptom), and most probably a whole series of other etiologies (10). The percent of patients with hypocretin deficiency in this group may depend on how carefully other potential causes for SOREMPs are excluded (see MSLT section).

IV. Idiopathic Hypersomnia with and Without Long Sleep Time

Similar to "narcolepsy", the term "idiopathic hypersomnia" had come to include many divergent meanings. In the European tradition, the term was reserved to include rare patients with a genuine "hypersomnia", meaning a report of sleepiness and increased sleep (12–14). Sleep drunkenness and other symptoms were typically associated.

In the United States, however, the term had evolved to include patients with unexplained excessive daytime sleepiness (rather than a genuine hypersomnia) but no SOREMP on the MSLT. To make the matter even more confusing, the school of Honda used the term "essential hypersomnia" to defined patients with unexplained daytime sleepiness but not extended sleep independent of MSLT findings (11). This confusion was reflected in the ICSD -1, where patients with either or both unexplained sleepiness and increased sleep could be diagnosed as "Idiopathic Hypersomnia". Bassetti and Aldrich (15) eloquently pointed out that these conditions may simply represent a symptomatological spectrum with narcolepsy based on a review of 42 cases.

To resolve these conflicting definitions, idiopathic hypersomnia was separated into two separate conditions (Table 2). The first condition, idiopathic hypersomnia "with long sleep time" corresponds to idiopathic hypersomnia as described the European tradition (12–14). These patients are rare, have persistent prolonged nocturnal sleep ($\geq$10 hrs) that must be documented by interview or sleep logs. Nocturnal polysmonography also indicates long sleep time and no other sleep disorders. During the day, patients are still sleepy, and it may even be difficult to conduct or interpret the MSLT. In cases with normal MSLT findings, long-term sleep monitoring or actigraphy may be used to document excessive amounts of sleep. One or no SOREMPs is observed if a MSLT is conducted.

This rare condition is to be contrasted with idiopathic hypersomia "without long sleep time". This condition is not a true hypersomnia, as daily sleep is not increased. It is characterized by normal sleep, unexplained sleepiness and MSLT findings indicative of sleepiness. Habitual sleep time and sleep time during polysomnography must be longer than 6 hrs to exclude sleep deprivation. Idiopathic hypersomnia without long sleep time may be difficult to distinguish from narcolepsy without cataplexy or from insufficient sleep in long sleepers.

V. Need for Future Studies

We believe that the revised ICSD-2 will be easier to use than the ICSD-1 and will lead to more homogenous patient groups etiologically. This is most likely to be the case for narcolepsy-cataplexy cases and idiopathic hypersomia with long sleep time. To date, however, only the category of narcolepsy-cataplexy is justifiable as a disease entity based on its established etiology. The cause of the other conditions is unknown, and the separation proposed somewhat artificial, reflecting clinical insight, historical work and practical aspects in the diagnosis of these conditions. The revised classification will need to stand the test of time and use in clinical sleep centers. This, together with the acute need to better understand what could confound the MSLT, must be the object of urgent future research.

References

1. American Academy of Sleep Medicine. ICSD-2. International Classification of sleep disorders, 2nd ed.: Diagnostic and coding manual. Westchester, Illinois: American Academy of Sleep Medicine, 2005, pp 297.

2. American Sleep Disorders Association. ICSD-1. The International Classification of sleep disorders, revised: Diagnostic and coding manual. Rochester, Minnesota: American Sleep Disorders Association, 1997, pp 401.
3. Carskadon MA, Dement WC, Mitler MM, Roth T, Westbrook PR, Keenan S. Guidelines for the multiple sleep latency test (MSLT) a standard measure of sleepiness. Sleep 1986; 9(4):519–524.
4. Littner MR, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, Hirshkowitz M, Daniel LL, Bailey D, Berry RB, Kapen S, Kramer M; Standards of Practice Committee of the American Academy of Sleep Medicine. Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test. Sleep 2005; 28(1):113–122.
5. Moscovitch A, Partinen M, Guilleminault C. The positive diagnosis of narcolepsy and narcolepsy's borderland. Neurology. 1993; 43(1):55–60.
6. Aldrich MS, Chervin RD, Malow BA. Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep. 1997; 20(8):620–9.
7. Carskadon MA, Dement WC. Effects of total sleep loss on sleep tendency. Percept Mot Skills 1979; 48:495–506.
8. Roehrs T, Zorick F, Sicklesteel J, Wittig R, Roth T. Excessive daytime sleepiness associated with insufficient sleep. Sleep 1983; 6: 319–325.
9. Chervin RD, Aldrich MS. Sleep onset REM periods during multiple sleep latency tests in patients evaluated for sleep apnea. Am J Respir Crit Care Med. 2000; 161(2 Pt 1):426–31.
10. Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Archives of Neurol 2002; 59:1553–1562.
11. Honda Y, Juji T, Matsuki K, Naohara T, Satake M, Inoko H, Someya T, Harada S, Doi Y. HLA-DR2 and Dw2 in narcolepsy and in other disorders of excessive somnolence without cataplexy. Sleep 1986; 9:133–142.
12. Roth B. Narcolepsy and hypersomnia: review and classification of 642 personally observed cases. Schweiz Arch Neurol Neurochir Psychiat 1976; 119: 31–41.
13. Roth B, Nevsimalova S, Rechtschaffen A. Hypersomnia with sleep drunkenness. Arch Gen Psychiat 1972; 26: 456–462.
14. Billiard M, Dauvilliers Y. Idiopathic Hypersomnia. Sleep Med Rev. 2001; 5(5):349–358.
15. Bassetti C, Aldrich MS. Idiopathic hypersomnia. A series of 42 patients. Brain. 1997; 120(Pt 8): 1423–1435.
16. Bassetti C, Gugger M, Bischof M, et al. The narcoleptic borderland: A multimodal diagnostic approach including cerebrospinal fluid levels of hypocretin-1 (orexin A). Sleep Med 2003; 4: 7–12.

Appendix A
Guidelines for the Appropriate Use of CSF Measurements to Diagnose Narcolepsy

LING LIN, CLAUDIO L. BASSETTI, GERT JAN LAMMERS, SEIJI NISHINO, MICHAEL H. SILBER, T. KANBAYASHI, JAMIE M. ZEITZER, MICHEL BILLIARD, SEBASTIAAN OVEREEM, YVES DAUVILLIERS, LAURENCE K. OLIVER, GEERT MAYER, and EMMANUEL MIGNOT

I. Introduction

A workshop on the use of CSF hypocretin-1 (Hcrt-1) measurements to diagnose narcolepsy was convened and included specialists and current users of the test. A concern of all participants was the increased use of the test without full understanding of its technical difficulties. A noted problem was the report of different mean normal values across testing centers, a difference due to across-center assay reliability of the radioimmunoassay (RIA). The committee also noted an alarming number of publications reporting on the inappropriate use of plasma orexin-A/Hcrt-1 measurement.

To solve this problem, the committee decided to establish a selected number of Centers of Excellence listed below. These centers will be used to provide guidance and reference samples to emerging laboratories wishing to set up the assay for diagnostic purposes. A detailed protocol for CSF Hcrt-1 measurements, together with a list of resource centers and important technical caveats, are listed below.

II. Hcrt-1 Radioimmunoassay (RIA): Principles and Pitfalls

The assay is based upon the competition of ^{125}I-hypocretin-1 and Hcrt-1 (either from standard or samples) binding to the limited quantity of anti-hypocretin-1 antibodies in each reaction mixture. As the quantity of Hcrt-1 standard or samples in the reaction increases, the amount of ^{125}I-peptide able to bind to the antibody is decreased. By measuring the amount of ^{125}I-hypocretin-1 bound as a function of the concentration of peptide in standard reaction mixtures, it is possible to construct a "standard curve" from which the concentration of Hcrt-1 in unknown samples can be determined (Fig. 1). The detection limit (sensitivity) of an assay is typically defined by the IC90 or IC95. This value is the hypocretin concentration value where a decrease of 5–10% of the radioactivity signal is detected (difference between no cold ligand present and a very low concentration of cold ligand present, see Figure 1). Below the IC90-IC95 value, it is commonly extremely difficult to accurately measure concentrations, as

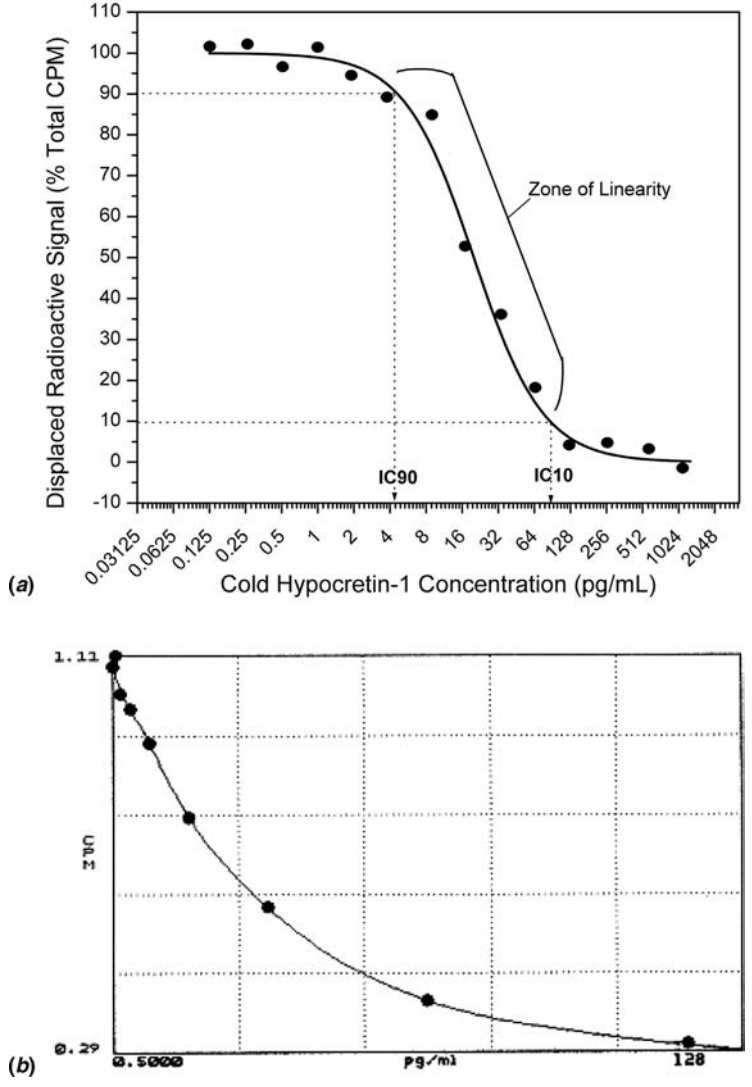


Figure 1 Typical standard curves for the assay. (***a***) Typical curve obtained over a very large range of applied Hcrt-1. (***b***) Curve obtained with Hcrt-1 standard amountranging from 1 pg/ml to 128 pg/ml, as usually applied in the assay. As the concentration of cold standard increases, binding of ^{125}I-hypocretin to the antibody is displaced and the resulting captured signal is decreased. Note that the signal only starts to decrease around 4–8 pg/ml, indicating the limit of detection for this particular assay. Very small signal differences (Y-axis) are detected below this value and will be sensitive to assay background noise. Similarly, there is little measured signal difference (Y-axis) between high and very high hypocretin value (for example, 64 and 128 pg/ml), thus Hcrt-1 concentration above 128 pg/ml will not be well measured. Reliable concentrations are only obtained in the linear portion of the curve, between 8 and 128 pg/ml.

very small changes in signal will result in large changes in estimated Hcrt-1 concentration (Figure 1). The same problem also occurs at the other end of the spectrum, when high Hcrt-1 concentrations, for example above the IC10, are applied (Figure 1).

Typical detection limit for this assay with the commercially available antibody and radioligand is usually 2-8 pg/tube. In the direct assay where 100 μl of CSF is applied, a value below 20-80 pg/ml may therefore appear as "undetectable". Importantly, however, significant differences in the detection limit are often observed across assays. Intra-assay reliability is high (<5%), but interassay reliability may vary of up to 20%, most probably because of variability in antibody batch quality, purity of the peptide and specific activity (counts per minute per one mole of hypocretin) of the labeled ^{125}I-hypocretin ligand.

Samples are always measured in duplicates. To reduce interassay variation, we recommend that all users of the test prepare a large amount of a standard CSF sample (*e.g.*, 50 ml of pooled cisternal CSF, see internal standard section), and aliquot it into single-run volume, then freeze and store at −80 °C. An aliquot of this sample can then be sent to Stanford University or another listed Center of Excellence (please contact the center first to coordinate receipt of the sample). The corresponding center will send back a concentration value that can be used to calibrate CSF Hcrt-1 values locally. Alternatively, one of the reference centers (G.J. Lammers), has kindly offered to provide large amounts of pooled CSF of known Hcrt-1 concentration. When a sufficient number of runs have been made to establish a mean value with this standard, then a conversion factor can be calculated to calibrate reference center values. A standard CSF sample with known calibrated Hcrt-1 value should be used in all assays to correct values based on the results of this measurement to reduce interassay variability. An aliquot of known CSF Hcrt-1 concentration will also be sent by one (GJ Lammers) of us to all reference centers on a yearly basis to ensure quality control over time.

Using this technique, a value below 110 pg/ml is diagnostic for narcolepsy, providing the result is interpreted together with the clinical context (see other chapters in this book). Samples with values close to the detection limit for a given assay (i.e., 20–80 pg/ml depending on the specific assay, see above) should be reported as "undetectable." Assays with unusually high detection limit (>80 pg/ml) or with a noisy standard curve should be repeated. Samples with a difference of duplicate values above 15% should be re-evaluated. If samples are not exchanged to normalize values with other centers, it is also possible to measure CSF hypocretin in the same assay in 10 controls and consider values in the narcolepsy range when they are below 30% of the mean control value obtained in the assay. This however produces some confusion in the literature, so we strongly advise calibration of values with the listed reference centers.

III. Lumbar Puncture and Stability of CSF for Hcrt-1 Assays

Lumbar punctures are performed according to local guidelines. At Stanford University, we advise the use of a double-needle lumbar puncture (LP) technique, as this reduces considerably the risk of post-LP headaches. The double needle consist of a 3.81 cm, 20 gauge spinal needle used as a guide needle, and an 8.89 cm 24 gauge Whitacre needle. A minimum of 1 ml is needed, but up to 8 ml may be drawn. We also advise rapid

freezing CSF on dry ice, although current data suggest stability of hcrt-1 at room temperature for extended periods of time.

It is essential to warn patients of potential risks and follow country-specific regulations for a test that is still considered a research tool. The lumbar puncture procedure can acutely cause some pain and discomfort. In 5% to 20% of the cases, headaches can be observed in the 10–12 hours following the procedure. It is important to tell the patients that these headaches are typically long lasting (days) and generally positional (when standing).

IV. Hcrt-1 Radioimmunoassay (RIA) Protocol

A. Materials

- RK-003-30 Orexin A (Human, Rat, Mouse), RIA Kit. Phoenix Pharmaceuticals 530 Harbor Boulevard Belmont, CA 94002 USA Tel: 650-610-8883 or 800-988-1205 Fax: 650-610-8882 E-mail: info@phoenixpeptide.com; one kit is sufficient to measure CSF Hcrt-1 in duplicate approximately 50 samples. The kit should be kept frozen and placed at 4°C several hours (or 12–24 hr) prior to using.
- A refrigerated (4°C) centrifuge able to run at 3500 rpm (2799 RCF max), equipped with a swing-out rotor and buckets accommodating 5 mL centrifugation tubes.
- Pipettes 5–50 μl and 20–200 μl. Disposable sterile pipette tips 1–200 μl.
- 12*75 mm Borosilicate Glass tubes from Fisher Scientific. Cat # 14-961-26 or Corning Cat # 99445-12.
- Frozen aliquots (250 μl) of CSF samples, to be used as an internal control.

B. Assay Day 1

Dilute the RIA buffer (50 ml, from kit) with 150 ml of distilled water (now you have a total 200 ml RIA buffer ready to use). This buffer is used to reconstitute all of the other compounds in the RIA kit. Of note, the diluted buffer can be stored at 4°C and reused for up to a month.

Add 1 ml of RIA buffer to the standard Peptide tube; leave the tube at room temperature for 5 minutes and vortex well (make sure the lyophilized powder is completely dissolved). Note: the left over standard solution can be stored at 4°C and reused for up to a month. Note: Before adding buffer, carefully examine the Eppendorf tube containing the standard. During shipping, part of or the entire lyophilized peptide may have come loose from the bottom of the tube and may be sticking to the cap or wall of the tube. Gently tap or centrifuge the tube to dislodge powder from the cap or wall. Carefully open the tube and add buffer.

Reconstitute the rabbit anti-peptide serum (anti-hypocretin-1 antibody) with 13 ml of RIA buffer, mix well and keep on ice. Note: the left over antibody solution can be stored at 4°C and used for up to one month.

Prepare peptide standard solutions as follows (all reactions should use 12*75 mm Borosilicate Glass tubes from Fisher). Defrost one tube of 15–20 μl stock of standard.

Tube	RIA buffer	Adding	Amount of standard/tube
0	990 μl	10 μl from stock	128 ng
A	990 μl	10 μl from tube 0	128 pg
B	500 μl	500 μl from tube A	64 pg
C	500 μl	500 μl from tube B	32 pg
D	500 μl	500 μl from tube C	16 pg
E	500 μl	500 μl from tube D	8 pg
F	500 μl	500 μl from tube E	4 pg
G	500 μl	500 μl from tube F	2 pg
H	500 μl	500 μl from tube G	1 pg

Set up initial RIA reaction as follow (all standard and sample reaction need duplicates):

1. Number duplicate tubes as NSB, TB, A, B, C, D, E, F, G, H for standards; number in duplicate 1,2,3, for your samples; number two tubes for your internal control (see note on last page).
2. Add 100 μL RIA buffer to TB tube. Add 100 μL standard solution to each standard tube from H to A.
3. Add 100 μL CSF sample to each appropriately marked tube, in duplicate.
4. Add 100 μL anti-hypocretin-1 antibody to each tube.
5. Cover with Saran Wrap, vortex, and keep at 4°C for 16–24 hours.

C. Assay Day 2

Reconstitute the ^{125}I-hypocretin-1 with 13 ml of RIA buffer and mix well to make tracer solution. Use a Gamma-counter to check a 100 μl aliquot of this concentration of tracer solution and adjust the count to 8,000–12,000 cpm.

If the count is much higher, dilute the tracer solution to bring final count between 8,000–12,000 cpm/100 μl. *Note*: The reconstituted ^{125}I-hypocretin-1 solution can be stored at 4°C, but should be used within a month.

Add 100 μl of the tracer solution to each tube.

Cover with Saran Wrap, vortex all tubes, and keep at 4°C for 16–24 hours.

D. Assay Day 3

Reconstitute the Goat Anti-Rabbit IgG Serum (GAR) with 13 ml of RIA buffer. *Note*: The remaining reconstituted GAR can be stored at 4°C, but should be used within a month.

Reconstitute the Normal Rabbit Serum (NRS) with 13 ml of RIA buffer. *Note*: The remaining reconstituted NRS can be stored at 4°C, but should be used within a month.

Add 100 μl of GAR to each tube.

Add 100 μl of NRS to each tube.

Cover with Saran Wrap then vortex all tubes; follow by incubating all tubes at room temperature for 90 minutes.

Add 500 μl cold RIA buffer to each tube.

Centrifuge all tubes at 3,500 rpm (1989 avg. RCF, 2799 max RCF) for 25 minutes at 4°C.

Carefully aspirate off all the supernatant without touching the pellet. *Note*: For best results, the supernatants should be immediately aspirated after centrifugation. If the pellet is allowed to sit for more than 15-30 minutes, it may become detached and make aspiration difficult. If this happens, respinning of the tube in the centrifuge will not help to reattach the pellet. It is essential to be careful not to aspirate any pellet.

Use a Gamma-counter to determine the CPM of the pellet. The Gamma-counter should be set to read each sample for 1–3 minutes (the longer the count time, the greater the precision).

The Gamma-counter should print a standard curve and compute your sample concentration (depending on the exact model of Gamma-counter). Your final result should then be corrected using the internal control value of the reference CSF sample for each assay.

E. Standards

As mentioned above, it is essential to have available an internal standard solution to be used in every assay to reduce interassay differences. To achieve this goal, three possibilities can be used.

1. Request aliquots of reference CSF samples from a reference center (G.J. Lammers). The samples can be used in every assay as internal controls.
2. Create your own CSF internal standard: If you have extra CSF from several lumbar samples, they can be pooled together and then aliquoted to 230 μl/tube and kept at −80°C. Alternatively, it is possible to contact the neurosurgery department and ask them to provide a large amount of CSF (e.g., 50 ml) from patients subjected to ventricular drains. In these cases, individual patient samples should be first tested individually, as in some cases CSF Hcrt-1 can be very low. The samples can then be pooled into one or two master samples (if two, select samples with low level and high levels). In such pools, it is also better to avoid including bloody (pink) samples. The pooled sample should be tested for protein, glucose, erythrocytes and leucocytes to verify these values are within the normal range. The pooled samples can be sent to one of the reference laboratories or to Stanford for evaluation. Use one tube (or two tubes if high and low values samples are created) for each assay. A value will be provided back and can be used to correct all measurements. The pooled sample can then be aliquoted and used locally in every assay as an internal standard.
3. Use one of your peptide solution standards as internal control. For example, make 10 ml of the 160 pg/ml standard (the final concentration value for this internal control will be 16 pg/ml); aliquot this to 230 μl/tube and keep them at −80°C. Use one tube for each assay and this 10 ml internal control can be used for 40 assays. Importantly, however, the peptide solution control does not contain all the natural components of CSF (only hypocretin). It is thus generally recommended to rather used pooled CSF references if available (options 1 and 2).

F. Storage and Other Tips

Solutions (RIA buffer, anti-hypocretin-1 antibody, ^{125}I tracer) can be kept at 4°C but should be used within a month. ^{125}I usually expires after one month. The degradation products do not interfere with the assay, but the reduction in total radioactive count increases imprecision at low count levels.

It is better to design your experiment before you order the kit. One kit can measure more than 100 samples, if you do them all at once. Even though you only want to measure one sample, you still need a whole set of standards.

Hcrt-1 cannot be measured in blood using this method as the concentration of Hcrt-1 in blood is likely lower than the detection limit of this assay.

G. References Centers

- Emmanuel Mignot, Ling Lin, Seiji Nishino
  HHMI Stanford University Center for Narcolepsy, 701 Welch Road B, Basement, Room 145, Palo Alto CA 94304-5742, USA
  Phone: 650-725-6517, FAX: 650-725-4913
  Email contact: linglin@stanford.edu
- Claudio Bassetti
  Leiter Poliklinik und Chefarzt-Stv., Universitatsspital Zurich, Frauenklinik-strasse 26, 8091 Zurich-Schweiz, Switzerland
  Telephone: (41) 1-255-5503 or (41) 1-255-5502
  Fax: 41-1-255-4649
  Email: claudio.bassetti@usz.ch
- Michael Silber, Lawrence K. Oliver
  Sleep Disorders Center or Department of Laboratory Medicine, Mayo Clinic College of Medicine, 200 First Street SW, Rochester MN, 55905, USA
  Telephone: 507-284-1588
  Email: msilber@mayo.edu
- Gert Jan Lammers
  Leiden University Medical Center, Neurology Department, PO Box 9600, 2300 RC Leiden, The Netherlands
  Telephone: (31) 71-5262895; Fax: (31) 71-5248253
  Email: g.j.lammers@lumc.nl
- T. Kanbayahsi
  Department of Neuropsychiatry, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-0853, Japan
  Telephone: (81) 18-884-6122; Fax: (81) 18-884-6445
  Email: takahsik@psy.med,akita-u.ac.jp

H. Accreditation Procedures

Any new centers wishing to measure CSF Hcrt-1 should first establish pooled CSF samples to share with another center. These will be used to calibrate their reference values. A series of 10 control and 3 narcolepsy samples should then be exchanged and if values obtained are within a 10% variation, the center will be accredited to receive other samples. Samples can be shared at a later date if a problem occurs. Of note, this procedure is only voluntary and was established to facilitate scientific

exchange; most clinicians do not appreciate the relative values of RIA readings and are confused if different normative values are reported.

V. Suggested Reading

Bloom SR, Long RG. Radioimmunoassay of gut regulatory peptides: Prager, 1982.

Appendix B
Treatment Guidelines

MICHEL BILLIARD, CLAUDIO L. BASSETTI, JED BLACK, ROGER J. BROUGHTON, YVES DAUVILLIERS, YUTAKA HONDA, T. KANBAYASHI, GERT JAN LAMMERS, GEERT MAYER, EMMANUEL MIGNOT, JACQUES MONTPLAISIR, THOMAS POLLMÄCHER, THOMAS E. SCAMMELL, MICHAEL H. SILBER, KARL E. SONKA, and MICHAEL THORPY

I. Introduction

A workshop on guidelines for the treatment of narcolepsy was held within the framework of the 5th International Symposium on Narcolepsy in Monte Verita, Ascona.

Present management of narcolepsy with or without cataplexy relies on several classes of drugs, namely stimulants for excessive sleepiness and irresistible episodes of sleep, antidepressants for cataplexy and other REM sleep related symptoms, hallucinations and sleep paralysis, and hypnosedative drugs for disturbed nocturnal sleep. In addition behavioral measures are of notable value. The present re-emergence of gamma-hydroxybutyrate, a unique endogenous neurotransmitter/neuromodulator, under the name sodium oxybate, as a treatment of the three symptoms, is likely to change prescription practices in narcolepsy. Besides these symptomatic treatments, new therapies are in preparation based on pathophysiological mechanisms, either evidenced or assumed.

II. Excessive Daytime Sleepiness and Irresistible Episodes of Sleep

First line treatment of excessive daytime sleepiness and irresistible episodes of sleep should rely on modafinil, 100–400 mg/day (1–4). According to a randomized placebo-controlled trial (5), a split–dose regimen, one dose in the morning and one dose at the beginning of the afternoon, is advisable to improve wakefulness significantly in the evening. In case of an afternoon nap, it is recommended to take modafinil before rather than after the nap, due to the delay in the onset of action. In a few cases the dosage should be increased up to 600 mg, Increased dosage above 600 mg is usually not advisable. There is little evidence to support co-administration of modafinil with other stimulant(s). The possibility of altered plasma levels of some drugs should be borne in mind, in relation with the induction of human hepatic cytochrome P450 enzymes by modafinil. This applies in particular to oral contraceptive pills, hence the need for

higher doses of ethinyloestradiol. Adverse effects include mainly headache, nervousness, nausea, and rhinitis. There is no evidence that tolerance develops to the effects of modafinil on excessive daytime sleepiness, although it has been reported in clinical practice. Similarly, it is generally accepted that modafinil has a low potential abuse. Teratology studies performed in animals do not provide any evidence of harm to the fetus. However modafinil is contraindicated in narcoleptic pregnant women due to insufficient clinical studies.

The alternative treatment could be sodium oxybate (not yet registered for excessive daytime sleepiness in Europe) (6–7). Starting dose is 4.5 g split in 2 nightly doses (at bedtime and 2 hours later). Dose can be increased by 1.5 g every two weeks, up to a maximum of 9 g/night. Adverse effects include nausea, dizziness, headache, and less frequently sleep walking, enuresis, vomiting, fatigue, nightmare, abdominal pain, diarrhoea, anorexia. No clinical data on exposed pregnancies is available. Thus sodium oxybate is not recommended during pregnancy.

In addition to either treatment, behavioral treatment is always advisable. It includes scheduled naps, fixed wake-up and regular sleep schedule. Up to now there is no sufficient evidence to recommend any number, duration and temporal placement of the naps.

Second line treatment should be considered either in case of adverse effects such as disabling headache or excessive nervousness or in case of insufficient or decreasing activity of the drug. In the first case methylphenidate SR 20 mg in the morning with two pulse doses of 5 mg methylphenidate (non-SR) at peak times of sleepiness and in the second case modafinil plus two pulses of 5 mg methylphenidate (non-SR) at peak times of sleepiness (8). Most frequent adverse effects of methylphenidate are nervousness and insomnia, followed by anorexia, headache, dizziness and dyskinesias. Other products used in some countries include mazindol (2–4 mg/day) (9) and selegiline (10–40 mg/day) (10–11). Both drugs have the advantage of being active on both excessive daytime sleepiness and cataplexy. However potential adverse effects of mazindol include dry mouth, nervousness, constipation, and less frequently nauseas, vomiting, headache, dizziness, tachycardia, excessive sweating and the use of selegiline is limited by potentially serious sympathomimetic adverse effects and interaction with other drugs such as triptans and serotonin specific reuptake inhibitors.

Third line treatments are amphetamines, dextro-amphetamine SR-10-15 mg in the morning with 5 mg pulse at peak times of sleepiness or methamphetamine (10–60 mg) (12–13). These drugs are generally considered as more potent than modafinil. Frequent adverse effects include irritability, hyperactivity, mood changes, headache, palpitations, sweating, tremor, anorexia, insomnia and the risk of tolerance. There is little or no evidence of abuse or addiction to these drugs in narcoleptic patients. Caution should be used before proceeding to these drugs given the potential for tolerance and more rarely for abuse.

III. Cataplexy and Auxiliary Symptoms, Hallucinations, and Sleep Paralysis

First line treatment is still tricyclic antidepressants for a majority of sleep specialists. The first use of tricyclics (imipramine) for treating cataplexy dates back to 1960 in Japan (14). Clomipramine seems to be the drug of choice (15). Imipramine and protryptiline are other alternatives. The initial dosage should be as low as 10 mg to avoid anticholinergic adverse effects including dry mouth, sweating, constipation, tachycardia,

weight increase, hypotension, difficulty in urinating and impotence. Development of tolerance after a couple of months has been mentioned (16). Moreover there is a risk, should the tricyclics be suddenly discontinued, of a marked increase in the number and severity of cataplectic attacks, a situation referred to as *rebound cataplexy* or even *status cataplecticus*.

The alternative treatment for cataplexy is sodium oxybate (registered both in the USA and in Europe), at a starting dose of 4.5 g split in two nightly doses (at bedtime and two hours later) (6–7,17). Dose can be increased by 1.5 g every two weeks, up to a maximum of 9 g/night.

Other treatments include several unregistered drugs, based, however, on no or few randomized, placebo controlled clinical trials. Despite the absence of any randomized placebo controlled clinical trial, the norepinephrine/serotonin reuptake blocker venlafaxine is widely used today, at a daily dose of 37.5–300 mg/day (18). Norepinephrine reuptake blockers are also used. Increased heart rate and blood pressure are potential adverse effects. Norepinephrine reuptake blockers are also used. Among them are atomoxetine 18–100 mg/day, qd or split bid, reboxetine (2–10 mg/day (19), and viloxazine (100–200 mg/day), not a very potent anticataplectic drug, but with a very low potential for adverse effects (20). Serotonin specific reuptake inhibitors, fluoxetine (20–60 mg/day) (21), fluvoxamine (50–100 mg/day) (15) or paroxetine (20–40 mg/day) are active on cataplexy. In comparison with tricyclics, higher doses are needed. Adverse effects are less pronounced than with tricyclics. They include CNS excitation, gastrointestinal upset, movement disorders and sexual difficulties. As previously pointed out mazindol (2–6 mg/day) (9) and selegiline (10–40 mg/day) (10–11) have the advantage of being active on both excessive daytime sleepiness and cataplexy. However they have limiting potential adverse effects.

There is no set behavioral treatment of cataplexy. However subjects should be recommended to avoid triggers as much as they can.

A. Poor Sleep

Benzodiazepines or non-benzodiazepine hypnotics may be effective in consolidating sleep. Unfortunately objective studies are lacking in the intermediate or long term. It is worth mentioning the improvement reported by some patients once on modafinil. Eventually sodium oxybate might be the best option. No clear behavioral treatment emerges in the case of poor sleep.

B. Parasomnias

Vivid and frightening dreams and REM sleep behavior disorders are the most frequently reported parasomnias in narcoleptic patients. As concerns the former there is no systematic study or no case reports available either with a pharmacological approach or with a behavioral approach. In the case of REM sleep behavior disorder the use of clonazepam was reported as successful in two occasions (22–23). Another possibility, based on an open label study, is melatonin at a dose of 3 to 12 mg per night (24).

C. Associated Clinical Features

The prevalence of obstructive sleep apnea/hypopnea is larger in narcoleptic patients than in the general population. One potential explanation is the frequency of an

increased body mass index in narcoleptic subjects. However there is no documented effect of continuous positive airway pressure in narcoleptic patients affected with obstructive sleep apnea/hypopnea syndrome.

The prevalence of periodic limb movements in sleep is also larger in narcoleptic patients than in the general population. This could be related to the dopaminergic impairment characteristic of narcoleptic patients. L-dopa, GHB and bromocriptine are effective. However there is no documented effect on excessive daytime sleepiness.

Depression is more frequent in narcoleptic patients than in the general population. Antidepressants and/or psychotherapy should be used in spite of any systematic study of these therapeutic procedures in narcoleptic patients.

D. Psychosocial Support and Counselling

Interaction with other narcoleptic patients and counselling from social workers are advisable.

IV. General Recommendations

A prerequisite before implementing a potentially life long treatment is to establish an accurate diagnosis of narcolepsy with or without cataplexy, and to check for possible co-morbidity. Following a complete clinical interview the patient should undergo an all-night polysomnography followed immediately by a multiple sleep latency test. HLA typing is not indispensable. CSF hypocretin-1 measurement is available in only a limited number of laboratories. It should be considered in some cases only, particularly if the multiple sleep latency test cannot be used or provides conflicting information. It should be remembered that levels of CSF hypocretin are only significantly reduced or absent in cases of narcolepsy with cataplexy. In the absence of cataplexy, the value of measuring hypocretin is debatable.

Once diagnosed, patients must be given as much information as possible about their condition (nature of the disorder, genetic implication, medications available and their potential adverse effects) to help them cope with a potentially debilitating condition.

Regular follow-up of patients, say every six months, is essential to monitor response to treatment, to adapt the treatments in case of insufficient response or adverse effects, to watch for weight gain and, above all, to encourage the patient to persist with a management plan.

Polysomnographic re-evaluation of patients should be considered in case of worsening of symptom(s) or development of other symptoms.

V. Future Treatments

Current treatments of human narcolepsy are symptomatically based. However given the major developments in understanding the neurobiological basis of narcolepsy, new therapies are likely to emerge.

There are three current focuses for future therapy:

- Symptomatic endocrine/transmitter modulating therapies: GHRH antagonists, GHB agonists, GABA-B agonists, and histamine agonists (selective H3-antagonists) all tried in narcoleptic mice or canines

- Hypocretin-based therapies: hypocretin agonists and cell transplantation
- Immune-based therapies including steroid therapy, intravenous-immunoglobulins (IVIg) and plasmaphoresis. The latter are of special interest since several attempts have been made so far in man, the most promising being an association of prednisone and IVIg near the onset of narcolepsy in a 10-year old boy (25) and IVIg alone in 4 subjects (26) and in another 4 subjects (27) with positive subjective effects on sleepiness and/or cataplexy.

VI. Conclusion

The recommendations expressed in this workshop are based on the best currently available scientific evidence and clinical experience. However developments in the field of narcolepsy are rapidly advancing. The use of sodium oxybate is already widespread in the USA and may become widespread too in Europe if it gets administrative approval. In the medium and long term, the development of etiologically oriented treatments might replace current symptomatic treatments.

References

1. Billiard M, Besset A, Montplaisir J, Laffont F, Goldenberg F, Weill JS, Lubin S. Modafinil: a double-blind multicenter study. Sleep 1994; 17(suppl): 107–112.
2. Broughton RJ, Fleming JAE, George CFP, Hill JD, Kryger MH, Moldofsky H, Montplaisir JY, Morehouse RL, Moscovitch A, Murphy WF. Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. Neurology, 1997; 49: 444–451.
3. U.S. Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil for the treatment of pathological somnolence in narcolepsy. Ann Neurol 1998; 43:88–97.
4. U.S. Modafinil in Narcolepsy Multicenter Study Group. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy. Neurology 2000; 54:1166–1175.
5. Schwartz JR, Feldman NT, Bogan RK, Nelson MT, Hughes RJ. Dosing regimen of modafinil for improving daytime wakefulness in patients with narcolepsy. Clin Neuropharmacol 2003; 26:252–257.
6. U.S. Xyrem® Multicenter Study Group. A randomized, double-blind, placebo-controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. Sleep 2002; 25:42–49.
7. US Xyrem® Multicenter Study Group. A 12-month, open-label multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. Sleep 2003; 26:31–35.
8. Mitler MM, Shafor R, Hajdukovik R, Timms RM, Browman CP. Treatment of narcolepsy: objective studies on methylphenidate, pemoline and protriptyline. Sleep 1986; 9:260–264.
9. Shindler J, Schachter M, Brincat S, Parkes JD. Amphetamine, mazindol and fencamfamin in narcolepsy. BMJ 1985; 290:1167–1170.
10. Hublin C, Partinen M, Heinonen E, Puukka P, Salmi T. Selegiline in the treatment of narcolepsy. Neurology 1994; 44:2095–2101.
11. Mayer G, Meier-Ewert K. Selegiline Hydrochloride treatment in narcolepsy. A double-blind, placebo-controlled study. Clin Neuropharmacol 1995; 18:306–319.
12. Mitler MM, Hajdukovic R, Erman M, Koziol JA. Narcolepsy. J Clin Neurophysiol 1990; 7:93–118.
13. Mitler MM, Hajdukovic R, Erman M. Treatment of narcolepsy with methamphetamine. Sleep 1993;16:306–317.
14. Akimoto H, Honda Y, Takahashi Y. Pharmacotherapy in narcolepsy. Diseases of the Nervous System. 1960; 21:1–3.
15. Schachter M, Parkes JD. Fluvoxamine and clomipramine in the treatment of cataplexy. J Neurol Neurosurg Psychiatry 1980; 43:171–174.

16. Guilleminault C, Raynal D, Takahashi S, Carskadon M, Dement WC. Evaluation of short-term and long-term treatment of the narcolepsy syndrome with clomipramine hydrochloride. Acta Neurol Scand 1976; 54:71–87.
17. U.S. Xyrem® Multicenter Study group. Sodium oxybate demonstrates long-term efficacy for the treatment of cataplexy in patients with narcolepsy. Sleep Med 2004; 5:119–123.
18. Smith M, Parkes JD, Dahlitz M. Venlafaxine in the treatment of the narcoleptic syndrome. J Sleep Res 1996; 5(suppl.1):217.
19. Larrosa O, de la Llave Y, Barrio S, Granizzo JJ, Garcia-Borreguero D. Stimulant and anticataplectic effects of reboxetine in patients with narcolepsy. Sleep 2001; 24:282–285.
20. Guilleminault C, Mancuso J, Quera-Salva MA, Hayes B, Mitler M, Poirier G, Montplaisir J. Viloxazine hydrochloride in narcolepsy : a preliminary report. Sleep 1986; 9:275–279.
21. Langdon N, Bandak S, Shindler J, Parkes JD. Fluoxetine in the treatment of cataplexy. Sleep 1986; 9:371–372.
22. Schuld A, Kraus T, Haack M, Hinze-Selch D, Pollmacher T. Obstructive sleep apnea syndrome induced by clonazepam in a narcoleptic patient with REM-sleep behavior disorder. J Sleep Res 1999; 8: 321–322.
23. Yeh SB, Schenck CH. A case of marital discord and secondary depression with attempted suicide resulting from REM sleep behavior disorder in a 35-year-old woman. Sleep Med 2004; 5:151–154.
24. Boeve BF, Silber MH, Ferman TJ. Melatonin for treatment of REM sleep behavior disorder in neurologic disorders: results in 14 patients. Sleep Med 2003; 4:281–284.
25. Lecendreux M, Maret S, Bassetti C, Mouren MC, Tafti M. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old boy. J Sleep Res 2003; 12:347–348.
26. Dauvilliers Y, Carlander B, Rivier F, Touchon J, Tafti M. Successful management of cataplexy with intravenous immunoglobulins at narcolepsy onset. Ann Neurol 2004; 56:905–908.
27. Zuberi SM, Mignot E, Ling L et al. Variable response to intravenous immunoglobulin therapy in childhood narcolepsy. J Sleep Res 2004; 13(Suppl.1):828.

Index

AASM. *see* American Academy of Sleep Medicine (AASM)
AB. *see* automatic behavior (AB)
abnormal motor activity, 165–168
abnormal NREM sleep, 271
ACTH. *see* adrenocorticotropic hormone (ACTH)
actigraphy, 71–73, 251
  sleepiness, 251
active hypersomnia, 339
active sleep propensity (SPAS), 147
active wakefulness (AW), 196, 387
acute disseminated encephalomyelitis, 326–327
acute disseminated encephalomyelitis (ADEM), 455
  EDS, 326
  MS, 329
addiction
  hypocretins, 46–47
ADEM. *see* acute disseminated encephalomyelitis (ADEM)
adolescents, 67–74
  EDS, 68
adrafinil, 561
  chemical structure, 562
adrenocorticotropic hormone (ACTH), 468
affective disorders, 493–494
age, 63–66. *see also* specific type age group
  biphasic distribution of, 63–66
  MSLT, 64–66
aging. *see* elderly
agoraphobia, 13, 20
AHI. *see* apnea-hypopnea index (AHI)
alertness
  variations in, 200–203
alertness measurements
  daytime, 193–197
allostasis, 47
Alzheimer's disease, 323
American Academy of Sleep Medicine (AASM), 225, 655
  MSLT, 656
amino acid residues
  DQ, 423
amphetamine, 518
amphetamines, 493, 513–516, 672. *see also* cocaine-amphetamine-regulated-transcript (CART)
  chemical structure, 548
  clinical pharmacology, 521
  dopaminergic nerve terminal, 517
  modafinil, 547, 549
  molecular targets, 516–518
  narcolepsy, 274
  side effects, 522
ANCOVA tests
  SP, 116

anesthesia
  general, 444
anorexigenic proopiomelanocortin, 45
anthropometric data, 486, 490
anticataplectic, 51, 53, 531–534
antidepressants, 641–644
  mechanisms of action, 532–535
  novel, 641–644
  pharmacology, 531–532
  second generation, 541
  tricyclic
    cataplexy, 533–538
    chemical structure, 539
anxiety, 13
apnea-hypopnea index (AHI), 135
appetite, 460–461
  alterations, 461–463
arousal defects
  sleepiness, 269–270
arousal systems
  daytime, 204
arousal threshold
  hypocretins, 45–46
asbasia, 49
Asia, 128, 129–130
asthma, 483
atonia
  REM, 4
attention
  Sternberg test of, 503–504
attentional problems, 503–504
atypical narcolepsy
  HLA alleles, 419
Australia
  driving regulations, 483
autoimmune sleep, 451–456
  cell mediated immunity and cytokine levels, 453
  future, 455
  humoral immunity, 451–453
  hypocretin system, 451–453
  pathology studies, 455
autoimmunity
  HLA associated diseases, 419–422
automatic behavior, 133–148
automatic behavior (AB), 133
  NREM sleep, 264
  risk factors, 141–144
  sleepiness, 145
auxiliary tests, 73
AW. *see* active wakefulness (AW)

BAC. *see* bacterial artificial chromosome (BAC)
baclofen, 380, 381, 382
  REM sleep, 383
bacterial artificial chromosome (BAC), 238
basal forebrain cholinergic neurons, 348
bed rest, 172–174
  simulation of ongoing time, 179
  simulation of ongoing time in, 179
behavior
  automatic, 133–148
  food-seeking, 395
  Hcrt neuronal activity, 391
  sleepiness, 265
  Xyrem, 378
behavioral management, 28–29, 497–508
  cataplexy, 504
  coexistent sleep disorders, 507
  daytime symptoms, 499–503
  EDS, 29
  ISE, 29
  medical education, 508
  nighttime symptoms, 498
  obesity and nutrition, 505
  patient and family education and counseling, 508
  psychiatric problems, 504
  psychosocial impact and behavioral intervention, 506
  risk, 507
behavioral states
  instability, 272
  orexins, 399–402
benzodiazepines
  poor sleep, 673
  receptor studies
    canine narcolepsy, 236
birth seasonality, 446–447
blood glucose
  oral glucose, 461
BMI. *see* body mass index (BMI)
body composition, 459
body mass index (BMI), 459, 460
  EPS, 135
body weight, 459, 460
  alterations, 461–463

brain
  hypocretin-recipient areas, 359
  narcoleptic, 44
  sleep-wake disturbances, 338
bruxism, 491
bupropion, 526

caffeine, 527
  chemical structures, 514
candidate gene approach, 429–431
canine narcolepsy
  benzodiazepine receptor studies, 236
  breeds affected, 233
  cataplexy, 533–537
  cloning studies, 238–239
  discovery, 4
  Dobermans, 234
  EEG studies, 235
  electrophysiological
    characterization, 234
  genetic linkage, 238
  history, 231–240
  hypocretin, 646
  hypocretin deficiency, 239
  Labradors, 235
  MSLT, 235
  neurochemical studies, 235–236
  pathophysiology, 231–240
  pharmacological
    characterization of, 234
  pharmacological studies, 236–237
  receptor binding studies, 236
  systemic drug administration
    effects, 237
carbon monoxide intoxication, 445
CART. *see* cocaine-amphetamine-regulated-transcript (CART)
cataphoria, 19
cataplessa, 49
cataplexy, 7, 49–59, 68, 74, 98, 133–148, 531. *see also* narcolepsy-cataplexy
  animal studies, 55
  attacks, 70
  autonomic changes, 157
  auxiliary symptoms, 673–674
  behavioral interventions, 504
  canine narcolepsy model, 533–537
  clinical aspects, 152–153
  clinical characteristics, 49–52
  comparison, 261
  continuous attacks, 53
  CSF, 67
    hypocretin, 58
    levels, 59
  daytime variations, 193–207
  definition of, 151
  development, 435
  drop attacks, 51
  drowsiness, 202
  EDS, 50, 54, 56, 58, 99
  EEG patterns, 195
  EMG registration, 156
  emotional triggers, 153
  environmental factors, 445
  eye movement patterns, 195
  food-elicited
    narcoleptic dogs, 406
  hallucinations, 203, 673–674
  Hcrt neuronal activity, 372
  HLA, 56, 57
  H-reflex studies, 158
  human narcolepsy model, 538–542
  human propagation, 263
  idiopathic hypersomnia, 98
  isolated, 100
  laughter, 152, 159
  medication, 51, 160, 272
  mice, 262
    characterization of, 258–263
    molecular genetic analysis, 258–269
  MSLT negative, 161
  narcolepsy, 37, 50, 59, 280
  neuroanatomy, 153–154
  neurobiology, 153–155
  neurochemistry, 153–154
  neurophysiology, 53–55, 151–162
  orexin neuropeptide
    system, 257–276
  origin, 152
  pathophysiology, 158
  pharmacological treatment, 55–56, 538–541
  propensity, 160
  REM sleep, 55, 133, 261
  risk factors, 141–144
  secondary, 52–53
    posterior hypothalamus, 52
  sleep, 187

cataplexy (*Contd.*)
sodium oxybate, 104
chronic administration studies, 628
short-term administration studies, 628
SP, 673–674
spontaneous, 54
treatment, 26–27
guidelines, 671
triggers, 422
untreated patients' sleep, 206
wakefulness, 202
waking, 187
cataplexy-like phenomena, 159–160
cell mediated immunity
autoimmune sleep, 453
cell transplantation, 648
hypocretin (orexin), 648–649
central nervous system (CNS), 27
diseases
neuroimmune mechanisms, 324–326
stimulants, 27, 513–529
energy homeostasis, 408
cerebrospinal axis
paralysis, 20
cerebrospinal fluid (CSF), 36–37, 67, 226, 443
cataplexy, 59, 67
central penetration of hypocretin, 647
detection limit, 280
diurnal fluctuations, 289
food deprivation, 290
hypocretin, 49, 73, 102, 279–284
cataplexy, 58
determination, 279–280
diagnostic utility, 295–296
hypersomnia, 288
hypocretin release, 361–362
idiopathic hypersomnia, 85
levels, 294
measurements, 289, 296–297, 323
MSLT, 99
partial hypocretin cell loss, 294
Zurich patients, 282
hypocretin-1, 101–102, 663, 665
measurements, 663–670
narcolepsy, 37–38
production
increase, 284
psychotropic drug effects, 290
ChAT. *see* choline acetyltransferases (ChAT)
child birth, 445
children, 67–74
diagnosis, 71–73
EDS, 68
sleepiness, 253
choline acetyltransferases (ChAT)
IHC, 378
cholinergic neurons
basal forebrain, 348
cholinergic system
GHB, 602
chronic fatigue syndrome, 83
circadian aspects in, 171–182
circadian control
sleep, 270–271
wake, 270–271
circasemidian aspects, 171–172
clinical reports, 7–14
clinical spectrum, 104
clinical trials
modafinil, 571–574
CNS. *see* central nervous system (CNS)
CNV. *see* contingent negative variation (CNV)
cocaine-amphetamine-regulated-transcript (CART), 213, 402
coexistent sleep disorders, 506
comorbidity, 488–494
German Narcolepsy Register, 486–488
prospective study, 488
retrospective study, 488
complex event related potentials, 251
consciousness, 22
contingent negative variation (CNV), 198
continuous ambulatory EEG monitoring, 251
continuous positive airway pressure (CPAP), 221
cortical neurons, 349
Hcrt, 350
counseling, 508
psychosocial, 28–29, 674
CPAP. *see* continuous positive airway pressure (CPAP)
critical Down syndrome region, 452
CSF. *see* cerebrospinal fluid (CSF)

cytokines
  autoimmune sleep, 453

d-amphetamine, 518
daytime alertness measurements, 193–197
daytime arousal systems
  weakness, 204
daytime drowsiness, 203–207
  duration of periods, 194
daytime sleep, 137–138, 197
  narcolepsy symptoms, 137–138
daytime symptoms
  behavioral management, 499–503
daytime variations, 193–207
delayed sleep phase syndrome, 505
dementia with Lewy bodies (DLB), 323
demyelinating diseases, 324–325
depression, 493–494, 505
dextroamphetamine SR-10-15, 672
diabetes, 461
diagnosis, 32–33, 65, 97
  children, 71–73
  EDS, 249
  idiopathic hypersomnia, 81–83
  KLS, 93–94
diagnostic symptoms
  EDS, 138–141
disrupted sleep, 66
disturbed nocturnal sleep, 370
  sodium oxybate, 622–624
  treatment, 27–28
DLB. *see* dementia with Lewy bodies (DLB)
Dobermans
  canine narcolepsy, 234
  narcoleptic, 274
dogs. *see also* canine narcolepsy
  food-elicited cataplexy, 406
  hypocretin, 646
  treatment, 301
dominant human leukocyte antigen, 31
dopamine
  chemical structure, 548
  hypocretin system, 365
  TBI, 335
dopaminergic effects
  anatomical substrates, 521
dopaminergic nerve terminal
  amphetamines, 517
dopaminergic system
  GHB, 597–600
dopamine systems
  waking functions, 205
Down syndrome, 432
Down syndrome cell adhesion molecule precursor (DSCAM), 432
DQ gene, 412, 414
  amino acid residues, 423
driving, 479–482
  expert reports, 481–482
  patient records, 479–480
  performance evaluation, 477–478
  problems, 506
  regulations, 482–483
  simulator tests, 250
  treatment, 482
drop attacks
  cataplexy, 51
drowsiness
  cataplexy, 202
  daytime, 194, 203–207
  inertia, 202
  measurements, 193–207
  variations in, 200–203
DSCAM. *see* Down syndrome cell adhesion molecule precursor (DSCAM)
dysomnia
  nocturnal, 498–499

EDS. *see* excessive daytime sleepiness (EDS)
education and counseling, 508
EEG. *see* electroencephalography (EEG)
EGFP. *see* enhanced green fluorescent protein (EGFP)
EHS. *see* essential hypersomnia syndrome (EHS)
elderly, 65
  MSLT, 66
  sleepiness rates, 243
electroencephalography (EEG)
  canine narcolepsy, 235
  continuous ambulatory monitoring, 251
  narcoleptic patient, 195
  patterns, 195
  wakefulness, 194
electromyography (EMG)
  cataplexy, 156

EMG. *see* electromyography (EMG)
emotional shock, 444
encephalomyelitis
  acute disseminated, 454
encephalophathies, 321–322
endocrine disturbances
  hypothetical model, 467
energy homeostasis
  CNS mechanisms, 408
enhanced green fluorescent protein (EGFP), 380
enuresis, 491
environmental factors, 104, 442–448
  cataplexy, 444
  clinical data, 443–445
  epidemiological studies, 445–446
  excessive daytime sleepiness, 444, 445
  focused studies, 445–447
  future, 448
  infectious agents, 446–447
  mechanisms, 447–448
epilepsy, 13, 18, 493
  criterion, 18
Epworth Sleepiness Scale (ESS), 111, 112, 245
  BMI, 135
  MSLT differences, 244–245
  MWT differences, 244
  quality of life in SAS, 245
  relationship of cataplexy, 147
  self-reported narcolepsy, 139
  validity, 244
ERP. *see* evoked related potentials (ERPs)
ESS. *see* Epworth Sleepiness Scale (ESS)
essential hypersomnia syndrome (EHS), 33, 34
  EDS, 33
ethnic groups, 421
Europe, 125–129, 127
  driving regulations, 482–484
  modafinil, 561–567
evoked related potentials (ERPs)
  types, 198
excessive daytime sleepiness (EDS), 25, 34, 283, 428, 440, 435, 499–501
  adolescents, 68
  behavioral modification, 29
  cardinal test methods, 252
  cataplexy, 50, 56, 58, 99
  children, 68
  CNS stimulant, 27
  diagnostic criteria, 655–660
  diagnostic criteria for syndromes, 655–660
  diagnostic symptoms, 138–141
  EHS, 33, 34
  environmental factors, 444, 445
  episodes of sleep, 671–672
  etiology, 243–244
  familial, 428
  familial history, 428
  families with narcolepsy, 31
  family history, 102
  future studies, 660
  GHB, 376
  idiopathic hypersomnia, 659
  management of, 220
  MS, 314
  MSLT, 246
  napping schedule, 29
  narcolepsy without cataplexy, 657–658
  narcoleptic, 34–35
  non-narcoleptic, 97
  pathophysiology, 243–244
  prepubertal narcolepsy, 63
  psychosocial counseling, 28–29
  REM sleep relationship, 190
  risk of motor vehicle accident, 250
  SL, 313
  sleep disorders, 220
  sodium oxybate
    chronic administration, 628
    short-term administration, 626–628
  SOREM, 100
  SOREMPs, 65
  Stanford Sleep Clinic, 428
  symptomatic narcolepsy, 308
  symptoms
    criteria of, 33
    definition, 33
    secondary, 503–504
  TBI, 336
  treatment, 26, 671
eye movement patterns, 195

familial excessive daytime sleepiness, 428
familial narcolepsy, 100–101, 431
  EDS, 31
family, 33–35, 417
  education and counseling, 508
  idiopathic hypersomnia, 84
fatigability, 503
fatigue
  definition, 336
FDI. *see* first dorsal interosseous muscle (FDI)
fecal matter, 17
FECT. *see* food elicited cataplexy test (FECT)
feeding
  Hcrt neuronal activity, 391
  Hcrt neurons, 399–411
fencamfamin, 514
first dorsal interosseous muscle (FDI), 161
fluoxetine, 533, 673
fluvoxamine, 673
food
  deprivation
    CSF levels, 289
  elicited cataplexy
    narcoleptic dogs, 406
  hypocretin release, 363–367
  seeking behavior
    REM sleep, 375
food elicited cataplexy test (FECT), 234, 647
forebrain waking systems
  motor systems, 357

gamma-amino butyric acid (GABA), 28
  receptors
    Hcrt neurons, 382
    hypocretin system, 375–383
    sleep, 382
gamma-amino butyric acid B (GABA-B)
  agonists, 674
  CNS inhibition, 606–607
gamma-hydroxybutyrate (GHB), 28, 542–543, 583–609, 641. *see also* sodium oxybate
  agonists, 674
  binding sites, 588–589
  cataplexy, 608–609
  cellular actions, 588–596
  cellular neurophysiologic studies, 379–382
  cholinergic system, 602
  concentrations, 587–588
  dopaminergic system, 597–600
  EDS, 376
  endogenous pathways, 584
  formation, 585–586
  functional neuroanatomical studies, 378–379
  future directions, 382–383
  GABAB receptor activation, 590–595
  glutamatergic and GABAergic systems, 596–597
  growth hormone, 602–603
  Hcrt neurons, 380, 382
  hypocretin system, 376–382
  metabolism, 587–588
  neurochemistry, 585–586
  neuromodulator, 596–603
  noradrenergic system, 600
  opioid system, 602
  pharmacokinetics in humans, 604–606
  SWS, 376, 644
  in vivo studies, 376–378
GBS. *see* Guillain-Barré syndrome (GBS)
GCS. *see* Glasgow Coma Scale (GCS)
Gelineau, Jean-Baptiste, 1, 11, 12–14, 15–25
Gelineau reports, 15–24
Gelineau's description, 8–9
general anesthesia, 444
gene therapy, 648
  hypocretin (orexin), 648
genetic counseling, 509
genetic rescue
  narcolepsy-cataplexy, 273, 274
  narcolepsy-cataplexy phenotype, 275
genetics, 104, 411, 428–429
  canine narcolepsy, 238
  HLA association, 423
  idiopathic hypersomnia, 84
  orexin neuropeptide system, 275
genome-wide search, 38–40
German Narcolepsy Register
  comorbidity, 486–488
GH. *see* growth hormone (GH)
GHB. *see* gamma-hydroxybutyrate (GHB)
GHRH antagonists, 674

Glasgow Coma Scale (GCS), 341
glucose, 457
GPCR. *see* G-protein coupled receptor (GPCR) systems
G-protein coupled receptor (GPCR) systems, 650
growth hormone (GH), 468
  GHB, 602–603
Guillain-Barré syndrome (GBS), 283, 327–328, 452
  hypocretin deficiency, 326

hallucinations, 203, 505
  associated with SP, 116
  SP, 115
  vivid hypnagogic, 499
haplotype, 430
Hcrt. *see* hypocretin (Hcrt)
headache, 493
head injury, 444
head trauma, 447
hereditary hyperekplexia, 158
HH. *see* hypnagogic/hypnopompic hallucinations (HH)
high fever, 444
high performance liquid chromatography (HPLC), 287
histamine, 528
  agonists, 674
  sleep regulation, 645
histaminergic arousal system, 269
histaminergic tuberomammilary nucleus, 46
historical aspects of treatment, 25–29
HLA. *see* human leukocyte antigen (HLA)
home
  psychosocial problems, 475
homeostatic aspects, 172
homeostatic mechanisms
  NREM sleep, 271–272
homeostatic sleep regulation, 187–191
homosexuality
  sexual inversion, 13
hormonal abnormalities
  hypocretin/orexin deficient narcolepsy, 468
HPA. *see* hypothalamic-pituitary-adrenal (HPA) axis
HPLC. *see* high performance liquid chromatography (HPLC)
H1 receptor agonists, 528, 533, 608, 645
5-HT. *see* serotonin (5-HT)
human leukocyte antigen (HLA), 31, 130, 411–424
  alleles
    atypical narcolepsy, 419
    idiopathic hypersomnia, 419
  associated diseases
    recent autoimmune studies, 422–423
  association, 427
    genetic transmission, 423
    hypocretin cell loss, 450
    hypocretin deficiency, 419–422
    multiplex family cases, 416–419
  blood typing, 129
  dominant, 31
  DQB1*0602, 442, 443, 447, 451
  DRS, 507
  frequency changes, 32
  gene
    function, 413–414
    structure, 413–414
  haplotype segregation, 417
  hypocretin, 57
  hypocretin status, 36
  negative narcolepsy, 101–102
  negativity
    symptomatic narcolepsy, 103
  orexin, 57
  positively, 102
    hypocretin deficiency, 420
  positive multiplex family cases
    hypocretin deficiency, 419
  studies, 412
  typing, 72–73
    cataplexy, 56
    idiopathic hypersomnia, 85
human lumbar CSF, 289
humoral immunity
  autoimmune sleep, 445
hyperinosemia. *see also* idiopathic hypersomnia
  immunogenetic aspects, 31–34
hyperinsomnia
  menstruation-linked, 91
hyperkinetic syndrome, 493
hypersexuality, 92

hypersomnia, 656
active, 336
cerebral tumors, 319-320
clinical features, 336–338
CSF hypocretin measurements, 289-290
definition, 336
hypocretin status, 315–318
hypothalamic lesion, 324
ICSD-revised, 91
menstruation-linked, 94
MS, 314
neuroanatomical considerations, 338–339
neurochemical considerations, 340–341
neurophysiologic considerations, 339–340
passive, 339
pathophysiology, 338–341
pontine stroke, 337
poststroke, 338
recovery, 338
posttraumatic, 338
recovery of, 337
primary, 337
psychiatric disorders, 82
secondary, 338
hypnagogic/hypnopompic hallucinations (HH), 68, 79, 141–144, 370
napping, 145
nightmare-sleep hallucinations, 145
proportions of sleepiness, 138
REM sleep, 131
risk factors, 141–144
sodium oxybate, 624
treatment, 542
hypnosis, 19
novel, 641–644
sedative
narcolepsy-cataplexy, 644
hypocretin (Hcrt), 70, 161, 171, 211, 226, 369, 370–394, 429, 492, 525, 675
addiction, 46–47
agonists, 543– 544, 650
allelic variance, 436
arousal threshold, 45–46
autoimmune sleep, 445
canine narcolepsy, 239
cell loss, 304
HLA association, 650
understanding the cause, 239
cell transplantation, 648–649
central penetration, 647
cortical neurons, 350
cortisol changes, 367
CSF, 279–284, 281
deficiency, 171, 181, 429
state boundary dyscontrol, 191
deficient animals, 356
deficient mice, 266
REM sleep, 267
deficient murine
and canine narcolepsy, 646
deficient narcolepsy
historical data, 467–468
hormonal abnormalities, 467–470
neuroendocrine studies, 468
results, 469
discovery, 413
dopamine, 365
effects, 347–350
endogenous peptide ligands, 607
excitatory effect, 400
exogenously applied, 359–360
future studies, 239
GABA receptors, 375–383
gamma-hydroxybutyrate, 376–382
gene therapy, 648
HLA, 36, 57, 419–422
HPA axis, 366
importance, 348
injection, 364
intracisternal administration, 648
intranasal administration, 646–647
involvement discovery, 432
levels, 281
ligand, 440, 642–643
literature, 47
localization, 44
locomotion, 364
locus coeruleus, 356
measurement, 296
Medtronic time-release pump, 240
molecular characterization of, 211–216
monoaminergic cells, 357
motor activity relationship, 356
mutated narcoleptic dogs, 646
mutation, 437
narcolepsy, 355
narcolepsy-cataplexy, 272–274

hypocretin (Hcrt) (*Contd.*)
neurons, 340, 404, 407, 468
activity of, 408
adaptive behavior of, 406
afferent of, 404–407
behavioral correlates, 390
cataplexy, 392
characterization of, 351
co-localized factors, 403–404
daily variation of, 362
discharge pattern, 390
efferent, 400–404
electrophysiological identification, 387–388
feeding behavior, 391, 399–408
feeding regulation, 402–403
GABA receptors, 382
GHB, 380
humoral factors of regulation, 407
identification, 389
illustration, 215
lateral hypothalamus, 214
LHA, 403
limbic system, 394, 406
MCH, 402
metabolic information, 45
molecular characterization, 213–215
motor activity, 391–392
properties of, 380
psychotropic drugs, 394
QW, 395
rat profile, 387–395
regulation, 404
schematic drawing, 405
sleep, 388–391
spatial resolution, 362
stress, 392–394
wakefulness, 402
neuropeptide system, 257–276
cataplexy, 257–276
genetic model, 275
neurotransmission
defect, 281
norepinephrine, 365
normal behavior, 357
normal role of, 355–357
NREM sleep, 268
orexigenic, 355
patterns of wakefulness, 364
peptides, 45, 645–650
analogues, 649–650
potential therapeutic agents, 542–543
regulation of behavioral states, 399–402
release
CSF hypocretin, 361–362
diurnal fluctuation, 363
effects of stress, 363–367
manipulation of food take, 363–367
REM sleep, 266–269, 371
RIA, 663–664
protocol, 666–670
role of, 43–47, 154–155
screening, 438
sleep neurobiology, 359–371
sleep stage regulation, 368–369
state boundary dyscontrol, 191
stress, 46–47
system, 299
cortical neurons, 350
effects, 347–349
genes, 435–439
importance, 347
inflammatory process, 299
wakefulness, 349
waking functions, 205
technical aspects in CSF, 287–295
TMN, 361
wakefulness, 349
wake-promoting action of, 347–353
hypocretin pathology
narcolepsy, 302–304
hypocretin status
neurological conditions, 311–315
hypothalamic-pituitary-adrenal (HPA) axis
hypocretin, 366
hypothalamic tumor
narcolepsy-cataplexy case, 312
hypothalamus, 43
hypocretin studies, 212
lateral, 44
MCH studies, 212
posterior
secondary cataplexy, 52
REM sleep, 369

ICSD. *see* International Classification of Sleep Disorders (ICSD)
  classification, 660
  clinical features, 78–79
  CSF hypocretin levels, 85
  differential diagnosis, 81–83
  epidemiology, 78
  excessive daytime sleepiness, 659
  family pedigree, 84
  frontiers, 81
  genetic basis, 84
  historical background, 77–85
  HLA alleles, 413
  HLA typing, 85
  laboratory tests, 79–81
  long sleep times, 82, 659–660
  narcolepsy, 78
  pathophysiology, 83–84
  polysomnography, 80
  treatment, 84–85
idiopathic hypersomnia, 77–85, 98
  cataplexy, 98
idiopathic narcolepsy, 282
idiopathic Parkinson's disease (IPD), 283
IEMG. *see* integrated neck EMG (IEMG)
IHC. *see* immunohistochemistry (IHC)
illicit use
  sodium oxybate, 626
immune-based therapies, 675
immunogenetics, 31–41
  hyperinosemia, 31–34
immunohistochemistry (IHC)
  ChAT, 378
immunomodulation, 650–652
infectious agents, 446–447
insomnia, 498
insufficient sleep syndrome, 83
integrated neck EMG (IEMG), 393
International Classification of Sleep Disorders (ICSD), 221, 289, 655
  definitions, 658
  revised
    hypersomnia, 91
  revisions, 660
  2 task force, 656
internistic diseases, 492–493
intracisternal administration, 648
intralaminar thalamic nuclei neurons, 348–349
intranasal administration, 646–648
intravenous immunoglobulins (IVIg), 675
  prednisone, 651
intrinsic sleep disorders, 492
involuntary sleep episodes, 499–500
IPD. *see* idiopathic Parkinson's disease (IPD)
irresistible sleep episodes (ISE), 25
  behavioral modification, 29
  CNS stimulant, 27
  psychosocial counseling, 28–29
  regular napping schedule, 29
  REM, 27
  treatment, 26
ISE. *see* irresistible sleep episodes (ISE)
isolated cataplexy, 100
isolated sleep paralysis (ISP), 110
  HH, 144
  narcolepsy, 114
  night walking, 114
  sleep attacks, 114
  SP episodes, 113
ISP. *see* isolated sleep paralysis (ISP)
IVIg. *see* intravenous immunoglobulins (IVIg)

Kleine-Levin syndrome (KLS), 89–94
  clinical features, 91–92
  diagnosis, 93
  differential diagnosis, 93–94
  epidemiology, 89–91
  etiology, 92–93
  ICSD-revised criteria, 91
  pathophysiology, 92
  published cases, 90
  treatment, 94
KLS. *see* Kleine-Levin syndrome (KLS)
knee buckling, 50

Labradors
  canine narcolepsy, 235
Lammers trial
  sodium oxybate, 624–625
lateral hypothalamic area (LHA), 44, 404
  Hcrt neurons, 214
  orexin neurons, 403
laterodorsal tegmentum (LDT)
  REM sleep, 268

laughter
  cataplexy, 152, 159
L-desprenyl, 526, 672
LDT. *see* laterodorsal tegmentum (LDT)
learning deficits, 473–474
leisure
  psychosocial problems, 475
leptin, 462, 463, 467–468
levodopa
  TBI, 335
LHA. *see* lateral hypothalamic area (LHA)
limbic encephalopathy, 322
limbic system
  Hcrt neurons, 394
  orexin neurons, 406
linkage studies, 431–432
literature
  hypocretins, 47
locomotion, 364
  hypocretin tone, 366
  issue of directionality, 365
locus coeruleus (noradrenaline), 360
  Hcrt systems, 356
long sleep time
  idiopathic hypersomnia, 82
LP. *see* lumbar puncture (LP)
lumbar puncture (LP)
  CSF stability, 665–666
  HCRT-1 assays, 665

maintenance wakefulness test (MWT), 219–228, 248, 651
  assessment of treatment effects, 248
  data, 225
  definition, 224–225
  driving ability, 248
  EDS diagnosis, 249
  MSLT, 651
    comparison, 248–249
  reliability, 248
MAO. *see* monoamine oxidase (MAO)
MAO-A. *see* monoamine oxidase-A (MAO-A)
MAOI. *see* monoamine oxidase inhibitor (MAOI)
mazindol, 525–526, 672
MCH. *see* melanin-concentrating hormone (MCH)
MDA. *see* methylenedioxyamphetamine (MDA)
MDMA. *see* methyllendioxy-methamphetamine (MDMA)
mean sleep latency
  MSLT, 197
  test data, 223
medication
  side effects of, 29
Medtronic time-release pump
  hypocretin deficiency, 240
melanin-concentrating hormone (MCH), 211, 212–213, 353
  Hcrt images, 352
  molecular characterization of, 211
  neurons, 215, 353
  orexin neurons, 402
  Orx images, 352
memory problems, 503
menarche, 444
meningitis, 13
menstruation
  hyperinsomnia, 91
menstruation-linked, 89, 91, 92, 93
  treatment, 94
metabolism
  alterations, 461–463
methylenedioxyamphetamine (MDA), 514
methyllendioxy-methamphetamine (MDMA), 514
methylphenidate, 514, 515
  side effects, 522
mice
  cataplexy, 262
    characterization of, 258–263
    molecular genetic analysis, 258–269
  molecular genetic analysis, 258–269
  sleep studies, 259
microsleep, 500
Middle East, 130
military service, 129
modafinil, 522–525, 671–672
  adverse events, 577
  chemical structures, 514, 548, 563
  clinical publications, 563–564
  discovery and development, 561–562
  European experience, 561–567
  future directions, 578

modafinil (*Contd.*)
history, 562
indications, 575
long term efficacy, 576–578, 576–577
mechanisms of action, 547–555, 550–553
postmarketing surveillance, 578
safety, 576–578, 576–577
SSRI, 576
US experience, 571–579
molecular genetic model
narcolepsy-cataplexy phenotype, 274–276
monoamine oxidase (MAO), 57
monoamine oxidase-A (MAO-A), 430
monoamine oxidase inhibitor (MAOI), 514, 532, 541–542
monoaminergic cells
Hcrt deficiency, 357
monosymptomatic narcolepsy, 100
morbid sleep
degrees, 19
morbus sacer, 17
motoneurons
TMS, 157
motor activity
abnormal, 165–168
Hcrt neuronal activity, 391–392
PLMS, 165
PLMW, 165
motor systems
forebrain waking systems, 357
motor vehicle accident
EDS, 250
MS. *see* multiple sclerosis (MS)
MSLT. *see* multiple sleep latency test (MSLT)
multiple sclerosis (MS), 314, 324–326, 328, 329
ADEM, 329
multiple sleep latency test (MSLT), 51, 63, 79, 219–228, 246–248, 650, 656–657
age, 64–66
canine narcolepsy, 235
cataplexy, 144
CSF Hcrt, 293
deficiency, 99
CSF hypocretin, 295
diagnostic protocol development, 222
EDS, 256, 257
elderly, 66
hypersomnia, 657
isolation of use, 227
limitations, 247–248
mean sleep latency, 197
misuses, 657
negative, 160
cataplexy, 161
objective of, 246
origin, 222–223
patient cost, 296
reliability, 246
SOREMP, 166, 247
US, 656
validity, 223
multiplex families
proband-parent trios, 436–437
murine narcolepsy-cataplexy
sleepiness, 269–272
muscle weakness, 12
MWT. *see* maintenance wakefulness test (MWT)
myotonic dystrophy, 318

naps
EDS, 29
HH, 136
ISE, 29
NREM sleep, 199
REM sleep, 66, 199
scheduled, 500–501
SP, 136
nap zone, 205, 501
narcolepsy, 1–4, 14. *see also* canine narcolepsy; specific area or type
atypical
HLA alleles, 419
clinical data, 69
criteria, 429
description, 207
development, 435
epidemiology, 125–131
evolution, 204
familial, 31, 100–101, 431
forms, 105
functional relevance, 369–371
idiopathic, 282

narcolepsy (*Contd.*)
independent effects, 114
limitations in studying, 131
molecular mechanisms, 32
monosymptomatic, 100
neuropathological studies, 294
pathophysiological basis, 181
pathophysiologic spectrum, 104
posttraumatic, 447
prepubertal, 63
psychiatric, 103–104
simulation of ongoing time, 178
spectrum
pathophysiological models, 293
surviving cells, 302
symptomatic, 102–103, 337
HLA negativity, 103
without cataplexy, 98–100, 293
narcolepsy-cataplexy, 81, 82, 657–659
animal models, 276
CSF hypocretin, 292–293
genetic rescue, 273, 274
genetic rescue demonstration, 274
molecular genetic model, 274–276
murine
sleepiness, 269–272
orexin, 272–274
pharmacologic rescue, 275
sedative hypnotics, 644
therapeutic targets, 276
narcolepsy without cataplexy (NwC), 134
symptoms, 146
narcoleptic animals, 301
narcoleptic brains, 44
narcoleptic Dobermans, 274
narcoleptic dogs
food-elicited cataplexy, 406
hypocretin, 646
treatment, 301
narcoleptic mice
molecular genetic analysis, 258–269
narcoleptic symptomatology, 301
narcoleptic tetrad, 74
NARP. *see* neuronal activity-regulated pentraxin (NARP)
National Institute of Health (NIH), 228
National Institutes of Neurological Disorders and Stroke (NINDS), 228
NE. *see* norepinephrine (NE)
NEI. *see* neuropeptide glutamic acidisoleucineamide (NEI)
neurodegenerative disorders, 283
neuroendocrine studies
hypocretin/orexin deficient narcolepsy, 468–469
neuroinflammatory disorders, 283
neurological diseases, 493–494
neuronal activity-regulated pentraxin (NARP), 403
neuropeptide (NPY)
orexigenic, 45
neuropeptide glutamic acidisoleucineamide (NEI), 215
neuropeptide glycine-glutamic acid (NGE), 215
neurophenomenology
SP, 109–111
neurotransmitter systems, 1
new treatments, 641–652
NGE. *see* neuropeptide glycine-glutamic acid (NGE)
Niemann-Pick disease, 73
Niemann-Pick type C disease, 317–318
nightmares, 491, 499
sleep hallucinations
HH, 145
SP, 136
night sleep
simulation of ongoing time, 177
night walking
independent effects, 114
ISP, 114
SP, 112
NIH. *see* National Institute of Health (NIH)
NINDS. *see* National Institutes of Neurological Disorders and Stroke (NINDS)
nocturnal dysomnia, 498–499
nocturnal polysomnography, 221
SDB, 227
nocturnal sleep, 138–141
disturbed, 370
treatment, 27–28, 632–634
inertia
studies, 198
nodding off, 264

non-amphetamine
stimulants, 522–527
non-catecholaminergic stimulants, 644–645
non-HLA genes, 427–432
non-narcoleptic excessive daytime sleepiness, 97
non-rapid eye movement (NREM) sleep
AB, 264
abnormal, 271
duration, 272
episodes, 175
homeostasis, 188–189, 190
homeostatic mechanisms, 271–273
MSLT naps, 199
narcolepsy, 99
orexin function, 268
pressure
cataplectic attacks, 190
regulation, 182
waking, 377
noradrenaline, 360
Hcrt systems, 356
noradrenergic arousal systems, 269
noradrenergic system
GHB, 591
norepinephrine (NE), 532, 673
chemical structure, 539, 548
hypocretin system, 365
normal behavior
Hcrt, 357
novel antidepressants, 641–644
novel hypnotics, 641–644
novel stimulants, 641–644
NPY. *see* neuropeptide (NPY)
NREM. *see* non-rapid eye movement (NREM) sleep
nutrition, 505–506
NwC. *see* narcolepsy without cataplexy (NwC)

obesity, 8, 69, 370, 459, 460, 468–469, 492–493, 505–506
behavioral management, 505
obstructive sleep apnea (OSA), 8
obstructive sleep apnea/hypopnea syndrome (OSAHS), 576
opioid system
GHB, 602
oral glucose
blood glucose, 457
orexigenic neuropeptide, 45
orexin (Orx). *see* hypocretin (Hcrt)
orexin mice
behavioral state transitions, 266
origin, 15
Orx. *see* hypocretin (Hcrt)
OSA. *see* obstructive sleep apnea (OSA)
OSAHS. *see* obstructive sleep apnea/hypopnea syndrome (OSAHS)
OSLER. *see* Oxford Sleep Resistance test (OSLER)
Oxford Sleep Resistance test (OSLER), 250

Paced Auditory Serial Addition Task (PASAT), 200
paralysis
cerebrospinal axis, 20
paraneoplastic syndrome, 328
parasomnias, 488–492, 673, 674
Parkinson's disease (PD), 322–323
idiopathic, 283
paroxetine, 673
PASAT. *see* Paced Auditory Serial Addition Task (PASAT)
passive hypersomnia, 339
patient education and counseling, 508
PD. *see* Parkinson's disease (PD)
pedunculopontine tegmentum (PPT), 383
pemoline, 514
peptides
ligands, hypocretins, 407
preprohypocretin, 422, 424
performance problems, 503
perifornical-lateral hypothalamus (PFH-LH), 379, 387
periodic leg movements in sleep (PLMS), 165–168
motor activity, 165
physiopathology, 167–168
RBD, 168
periodic leg movements while awake (PLMW), 167
motor activity, 165
Persian Gulf War veterans, 446
personality changes, 68

PFH-LH. *see* perifornical-lateral hypothalamus (PFH-LH)
pharmacologic treatment, 25–28
phenylisopropylamine, 514
physiological sleepiness, 243
plasmaphoresis, 675
PLMS. *see* periodic leg movements in sleep (PLMS)
PLMW. *see* periodic leg movements while awake (PLMW)
polypeptides
  preprohypocretin, 453
polysomnography, 72
  idiopathic hypersomnia, 80
  nocturnal, 221
    SDB, 227
POMC. *see* proopiomelanocortin (POMC)
pontine stroke
  hypersomnia, 340
ponto-medullary stroke, 341
poor sleep, 673, 674
posterior hypothalamus
  secondary cataplexy, 52
poststroke hypersomnia, 335–343, 336–337
  recovery, 338
posttraumatic hypersomnia, 335–343, 337–338
  recovery of, 338
posttraumatic narcolepsy, 447
PPT. *see* pedunculopontine tegmentum (PPT)
Prader-Willi syndrome (PWS), 316–317
prazosin, 54
prednisone
  IVIg, 651
pregnancy, 445
preprohypocretin peptides, 422, 423
preprohypocretin polypeptides, 454
prepubertal narcolepsy
  EDS, 63
pre-REM years, 2
primary hypersomnia, 342
proband-parent trios
  multiplex families, 436–437
progressive supranuclear palsy, 317
proopiomelanocortin (POMC), 45
  anorexigenic, 45
psychiatric disorders, 103, 493–494
  behavioral management, 497
  hypersomnia, 82
psychiatric narcolepsy, 103–104
psychosocial counseling, 28–29, 674
  EDS, 28–29
  ISE, 28–29
psychosocial impact, 473–476
  behavioral management, 497
  improving, 498
psychosocial problems
  home, 475
  leisure, 475
  relationships, 476
  road accidents, 475
  school, 473
  sexual activities, 476
  work, 474–475
  work accidents, 475
psychotropic drugs
  CSF, 290
  Hcrt neuronal activity, 391
psycho-vigilance test (PVT), 250
pupillography, 250
PVT. *see* psycho-vigilance test (PVT)
PWS. *see* Prader-Willi syndrome (PWS)

quality receiver operating curve (QROC), 58
quiet wakefulness (QW), 196, 388
  Hcrt neurons, 375

radioimmunoassay (RIA)
  curves, 664
  principles, 663
  reliability, 663
raphe nucleus (serotonin), 360
rapid eye movement (REM) sleep, 54, 443
  abnormality evidence, 3
  atonia, 4, 14
  awakenings, 202
  baclofen, 377
  cataplexy, 55, 133, 261
  vs. cataplexy, 533
  changes, 267
  deprivation
    cataplectic attacks, 190
    SOREMs, 189

rapid eye movement (REM) sleep (*Contd.*)
discovery, 2
dissociation hypothesis, 154,
duration of, 174
duration of time, 180
EDS, 27
EMG, 2
food-seeking behavior, 395
generated imagery, 111
homeostasis, 189–190, 267
hypnagogic hallucinations, 131
hypothalamus, 363
induced motor paralysis, 109
ISE, 27
latencies, 370
night, 66
SP, 146
latency, 66
LDT, 275
MSLT naps, 199
naps, 66
narcolepsy, 176
narcoleptics, 54
nightmares, 489
orexin, 257–276
orexin function, 268
oscillator, 175
periods
sleep onset, 2–5
SOREMPS, 224
phenomena, 97
pressure
cataplectic attacks, 190
prevalence establishment, 3
pulse
RemOn-RemOff cells, 182
sleep regulation, 180
regulation, 182
RemOff, 176
RemOn, 176
sleep atonia
dissociated manifestations, 157
sleep behavior disorder, 8
sleep debt, 375
sleep paralysis, 109, 133
SOREM, 257
SOREMPs, 197, 313
SWA, 173
SWS, 368
symptoms, 38
timing, 174
wake episodes, 175
waking, 369
rapid eye movement sleep behavior disorder (RBD), 165, 168, 263, 491
PLMS, 168
Rasmussen's syndrome, 322
RBD. *see* rapid eye movement sleep behavior disorder (RBD)
reaction time tests, 249–250, 503
rebound cataplexy, 673
receptor loci
hypocretin ligand, 440
relationships
psychosocial problems, 476
REM. *see* rapid eye movement (REM) sleep
RemOn-RemOff cells
REM pulse, 182
restless legs syndrome (RLS), 166
RIA. *see* radioimmunoassay (RIA)
RLS. *see* restless legs syndrome (RLS)
road accidents
psychosocial problems, 476

scheduled naps, 500–508
school
psychosocial problems, 476
SCN. *see* suprachiasmatic nucleus (SCN)
Scrima trial
sodium oxybate, 625–626
SDB. *see* sleep-disordered breathing (SDB)
secondary cataplexy
posterior hypothalamus, 52
secondary changes, 70–71
secondary hypersomnia, 342
CSF hypocretin, 302
second generation antidepressants, 541
sedative hypnotics
narcolepsy-cataplexy, 644
seizures, 4, 104
selective noradrenaline reuptake inhibitor (SNRI), 27
selective serotonin reuptake inhibitor (SSRI), 27, 53
chemical structure, 531
plus modafinil, 576

selegiline (L-desprenyl), 526, 672
serotonin (5-HT), 360, 532
serotonin specific reuptake inhibitors, 673
sexual activities
  psychosocial problems, 476
sexual inversion
  homosexuality, 13
shift work sleep disorder (SWSD), 576
shock
  emotional, 444
single-family TDT study, 414–416
single nucleotide polymorphisms (SNPs), 36,
single-strand conformational analysis (SSCA), 430
sleep, 187. *see also* autoimmune sleep; irresistible sleep episodes (ISE); isolated sleep paralysis (ISP); non-rapid eye movement (NREM) sleep; rapid eye movement (REM) sleep
  attacks, 13, 196, 400
    characterization, 258–263
    comparison, 264
    independent effects, 114
    ISP, 114
    mice, 258
    SP, 112
  cataplexy, 187
  circadian control, 270–271
  cortical synchronization, 43
  daytime, 197
  debt
    multivariable associations with, 142
    REM sleep, 368
  deprivation
    CSF concentrations, 361
    hypocretin release, 361–362
  diary, 71
  disorders
    coexistent, 506
    evaluation of, 220
    international classification, 658
    intrinsic, 492
    management of, 220
  disrupted, 66
  disruption, 371
  disturbances
    multivariable associations with, 142
  episodes
    involuntary, 499–500
  features stimulation, 174–180
  GABA receptors, 382
  Hcrt neuronal activity, 388–391
  homeostasis, 187
  hypocretin, 359–371
  latency, definition, 222
  morbid
    degrees, 19
  neurologic condition effects, 291–292
  poor, 663, 664
  promoting neurons, 341–342
  propensity, 253
  psychiatric effect conditions, 291–292
  regulation, 427
    importance of histamine, 645
    REM pulse, 180
    two-process model, 188
  satiation, 498
  simulation of ongoing time, 177
  states
    fragmentation, 265
    regulation, 401
  untreated patients', 206
Sleep Apnea Syndrome, 166
sleep-disordered breathing (SDB), 134
  multivariable associations with, 143
  nocturnal polysomnogram, 227
sleepiness
  AB, 145
  actigraphy, 251
  arousal defects, 269–270
  assessment methods, 253
  assessment of, 244
  children, 253
  daytime
    narcolepsy symptoms, 137–138
  different states of existence, 197–199
  fragmented behavioral patterns, 265
  murine narcolepsy-cataplexy, 269–272
  rates
    elderly people, 243
    young adults, 243
  states, 203
sleeping dropsy, 19
sleep onset REM period (SOREMP), 64, 97, 154, 224, 309, 533, 646
  cataplectic attacks, 239
  CSF hypocretin, 293, 651

sleep onset REM period (SOREMP) (*Contd.*)
EDS, 65
MSLT, 166, 247, 659
narcolepsy, 193
nocturnal polysomnography, 317
REM sleep periods, 224
sleep onset REM-sleep (SOREMs), 187, 189
EDS, 100
REM sleep, 257
sleep paralysis (SP), 111–117, 133–148, 370, 499. *see also* isolated sleep paralysis (ISP)
ANCOVA tests, 116
clinical analysis, 109–116
hallucinoid experiences associated with, 116
napping, 136
narcolepsy, 112
neurophenomenology of experiences, 109–111
nightmares, 136
night walking, 112
REM, 109
REM latencies, 146
REM sleep, 133
risk factors, 141–144
self-reported, 138
sleep attacks, 112
sleep's relationship, 145
sodium oxybate, 624
treatment, 532
V-M hallucinations, 115
with wake-sleep disruption, 113
without cataplexy, 112, 115
sleep paralysis with wake-sleep disruption (SPD), 113
sleep-wake
behavior
fragmented, 271
cycle
homeostatic regulation, 216
disorders
TBI, 284
disturbances
brain damage, 338
treatment, 342–343
regulation patterns, 173
slow wave sleep (SWS)
GHB, 644
REM sleep, 368, 369
SNP. *see* single nucleotide polymorphisms (SNPs)
SNRI. *see* selective noradrenaline reuptake inhibitor (SNRI)
sodium oxybate, 272, 641. *see also* gamma-hydroxybutyrate (GHB)
cataplexy, 104
chronic administration studies, 628
short-term administration studies, 626–628
disturbed nocturnal sleep
short-term administration, 632–634
dosing, 635
drug interactions, 635
excessive daytime sleepiness
chronic administration, 632
short-term administration, 628–629
formal development, 625–633
hypnagogic/hypnopompic hallucinations, 634
illicit use and abuse potential, 637
Lammers trial, 624–625
long-term experience, 622
narcolepsy, 272
open-label work, 622
pharmacokinetics, 635
placebo-controlled trials, 623–624
safety, 634
Scrima trial, 623–624
sleep paralysis, 634
tolerability, 634
somnosis, 19
SOREM. *see* sleep onset REM-sleep (SOREMs)
SOREMP. *see* sleep onset REM period (SOREMP)
SP. *see* sleep paralysis (SP)
SPAS. *see* active sleep propensity (SPAS)
SPD. *see* sleep paralysis with wake-sleep disruption (SPD)
SSCA. *see* single-strand conformational analysis (SSCA)
SSRI. *see* selective serotonin reuptake inhibitor (SSRI)
SSS. *see* Stanford Sleepiness Scale (SSS)

Stanford Sleep Disorders Clinic, 3
  EDS, 428
Stanford Sleepiness Scale (SSS), 220, 245
  method application, 245–246
startle disease, 159
status cataplecticus, 53, 71, 673
Sternberg test of attention, 503–504
steroid therapy, 675
stimulants, 641–644
  future, 527
  novel, 641–644
story telling, 13
stress
  hypocretins, 46–47, 363–367, 392–394
stroke
  epidemiology, 335–336
  ponto-medullary, 341
strychnine arsenate, 21
subvigilance syndrome, 204
suprachiasmatic nucleus (SCN), 363, 470, 649
SWS. *see* slow wave sleep (SWS)
SWSD. *see* shift work sleep disorder (SWSD)
symptomatic cataplexy
  hypocretin status, 315–329
symptomatic endocrine/transmitter modulating therapies, 674
symptomatic narcolepsy, 102–103, 336
  with cataplexy, 307–318
  CSF hypocretin measures, 306
  definition, 308–314
  EDS, 326
  HLA negativity, 103
  with hypocretin deficiency, 307–329
  hypocretin status, 315–329
  hypocretin system, 324
  without hypocretin deficiency, 307–329
symptoms, 37, 131, 146, 301, 308–314, 321, 370
  behavioral management, 499–503
  daytime sleepiness, 137–138
  EDS, 138–141
    criteria of, 33
    definition, 33
    secondary, 503–504
  nighttime
    behavioral management, 498
    REM sleep, 38
    self-reported, 140

TBI. *see* traumatic brain injury (TBI)
TDT. *see* transmission disequilibrium test (TDT)
Tetrodotoxin (TTX), 380
thyroid stimulating hormone (TSH), 468
thyrotropin-releasing hormone (TRH), 645
TI. *see* tonic immobility (TI)
TMN. *see* tuberomammilary nucleus (TMN)
TMS. *see* transcranial magnetic stimulation (TMS)
TNF. *see* tumor necrosis factor-alpha (TNF-Alpha)
tonic immobility (TI), 159
transcranial magnetic stimulation (TMS), 156
  motoneurons inhibition, 157
transmission disequilibrium test (TDT), 438
  single-family study, 414–416
tranylcypromine, 514
traumatic brain injury (TBI), 284, 335
  cataplexy, 337
  dopamine, 341
  hypersomnia, 335
  levodopa, 340
  sleep-wake disorders, 284
treatments, 651
  assessment, 248
  cataplexy, 26–27, 55–56, 531–544, 671
  disturbed nocturnal sleep, 27–28, 622–624
  dogs, 303
  driving, 479
  EDS, 26, 671
  guidelines, 671–675
  historical aspects, 25–29
  hypnagogic hallucinations, 531
  idiopathic hypersomnia, 84–85
  ISE, 26
  KLS, 94
  menstruation-linked, 94
  MWT, 248

treatments (*Contd.*)
narcoleptic dogs, 303
new, 641–652
pharmacologic, 25–28
sleep-wake disturbances, 335–343
TRH. *see* thyrotropin-releasing hormone (TRH)
tricyclic antidepressants
cataplexy, 531–544
chemical structure, 539
tropical diseases, 11
TSH. *see* thyroid stimulating hormone (TSH)
TTX. *see* Tetrodotoxin (TTX)
tuberomammilary histaminergic neurons, 348
tuberomammilary nucleus (TMN), 46, 360
histaminergic, 46
tumor necrosis factor-alpha (TNF-Alpha), 36
twins, 100–101
type 2 diabetes, 461

Ullanlinna Narcolepsy Scale (UNS)
development, 119–123
ultradian, 171–182
urine, 17

venlafaxine, 533, 673
ventral tegmental area (VTA), 46, 360, 386
ventrolateral preoptic area, 347
vertigo, 13, 20
veterans
Persian Gulf War, 446
vigilance tests, 201, 243–253, 503
comparison, 249
visual problems, 504
vivid hypnagogic hallucinations, 499
VTA. *see* ventral tegmental area (VTA)

wakefulness, 187. *see also* maintenance wakefulness test (MWT)
active, 196
assessment of, 244
cataplexy, 187, 202
categorization, 201
circadian control, 270–271
EEG, 194
fragmentation, 265–266
homeostatic regulation, 188
hypocretin effects, 360–341
maintaining, 400
neurobiology, 547–548
NREM sleep, 377
orexin neurons, 402
quiet, 196, 388
Hcrt neurons, 395
regulation, 401
REM sleep, 175, 377
substages, 194
weight, 459, 460
alterations, 461–463
Wernicke's encephalopathy, 321–322
Westphal, Karl Friedrich, 10–13, 15–25
Wilkinson auditory vigilance tests, 196, 200, 201, 503
Wisconsin Sleep Cohort Study (WSCS), 134
work
psychosocial problems, 474–475

Xyrem
behavioral state affects, 379
effects of, 381
rats, 372

young adults
sleepiness rates, 243

Zurich patients
CSF hypocretin levels, 282

Pulmonary Disease

about the book . . .

Compiled by an international group of more than 40 authors, this reference book supplies an engaging and comprehensive review of the major topics and key issues associated with narcolepsy and hypersomnia. Spanning the latest advances in the field, this source covers current diagnostic procedures, genetic developments, explorations of animal models, new definitions and criteria, and improved epidemiological surveys to reflect the explosion of research in this evolving science.

This source provides a comprehensive review of the clinical features and diagnostic criteria for narcolepsy and hypersomnia...reviews the symptoms, etiology, and pathophysiology of these disorders, and hypersomnia conditions...promotes an improved understanding of these conditions, and discusses methods of diagnosis and management, and research for advances in prevention and control of these diseases...and presents a clear overview of medications currently utilized in patient care to manage symptoms of these conditions and assesses current and emerging treatment options.

about the editors . . .

CLAUDIO L. BASSETTI is Full Professor and Head of the Neurological Outpatient Clinic, Emergency Service and Sleep Laboratory, and Vice-Chairman of the Department of Neurology, University Hospital, Zurich. Prof. Bassetti is the author of over 150 publications in peer-reviewed journals, Editor-in-Chief of the *Swiss Archives of Neurology and Psychiatry*, and serves on the editorial boards of four other journals. He is board member of different scientific societies including the European Neurological Society, the European Sleep Research Society, and the World Association of Sleep Medicine. Dr. Bassetti is currently vice-president of the Swiss Neurological Society and of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology. Dr. Bassetti received the M.D. degree from the University of Basel, Switzerland. Dr. Bassetti's research interests reflect his areas of clinical expertise and have centered on the clinical, neurophysiological and pathophysiological issues related to stroke and neurological sleep-wake disorders.

MICHEL BILLIARD is honorary Professor of Neurology at the Medical School, University of Montpellier I, Montpellier, France, and former Head of the Department of Neurology B, Gui de Chauliac Hospital, Montpellier, France. Dr. Billiard specialized in sleep disorders medicine, in the Department of Pathophysiology of Nervous Diseases, Montpellier, France; in the Sleep Laboratory, Department of Psychiatry, Royal Edinburgh Hospital, Edinburgh, Scotland; and in the Sleep Disorders Clinic, Medical Center, Stanford University, California. Dr. Billiard's main scientific interest is in the field of hypersomnias of central origin. Dr. Billiard has served on the editorial boards of the main North American and European sleep journals. He was the President of the European Sleep Research Society (1996–2000) and the Chairman of the Sleep Disorders Scientific Panel of the European Federation of Neurological Societies (2002–2006). Dr. Billiard received the M.D. degree from the University of Montpellier, France.

EMMANUEL MIGNOT is Howard Hughes Medical Investigator, Professor of Psychiatry and Behavioral Sciences and Director of the Center for Narcolepsy, Stanford University School of Medicine, California. Dr. Mignot is the recipient of numerous research grants and honors including the Jacobeus price, the Spencer price, and awards from the National Sleep Foundation, the Sleep Research Society, the American Academy of Sleep Medicine, and the American College of Neuropsychopharmacology. He is the coauthor of more than 100 original scientific publications and serves on the editorial boards of scientific journals in the field of sleep disorders research, pharmacology, and biological psychiatry. Dr. Mignot is an active member of several professional and governmental organizations including the Institute of Medicine and National Institutes of Health advisory boards. He is a former chair of the National Center on Sleep Disorders Research and a past president of the Sleep Research Society. He practices sleep medicine and specializes in evaluating and treating patients with narcolepsy and hypersomnia. Dr. Mignot received the M.D. and Ph.D. degrees from Paris V and VI University, France.

Printed in the United States of America

Cover drawing courtesy Antoinette Anderegg

DK5943

ISBN 0-8493-3715-1

90000

9 780849 337154

informa
healthcare
www.informahealthcare.com

270 Madison Avenue
New York, NY 10016

2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK